THE RECENT HISTORY OF TUMOUR NECROSIS FACTOR (TNF)

The transcript of a Witness Seminar held by the History of Modern Biomedicine Research Group, Queen Mary University of London, on 14 July 2015

Edited by A Zarros, E M Jones, and E M Tansey

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WHAT IS A WITNESS SEMINAR?

The Witness Seminar is a specialized form of oral history, where several individuals associated with a particular set of circumstances or events are invited to meet together to discuss, debate, and agree or disagree about their memories. The meeting is recorded, transcribed, and edited for publication.

This format was first devised and used by the Wellcome Trust's History of Twentieth Century Medicine Group in 1993 to address issues associated with the discovery of monoclonal antibodies. We developed this approach after holding a conventional seminar, given by a medical historian, on the discovery of interferon. Many members of the invited audience were scientists or others involved in that work, and the detailed and revealing discussion session afterwards alerted us to the importance of recording 'communal' eyewitness testimonies. We learned that the Institute for Contemporary British History held meetings to examine modern political, diplomatic, and economic history, which they called Witness Seminars, and this seemed a suitable title for us to use also.

The unexpected success of our first Witness Seminar, as assessed by the willingness of the participants to attend, speak frankly, agree and disagree, and also by many requests for its transcript, encouraged us to develop the Witness Seminar model into a full programme, and since then more than 60 meetings have been held and published on a wide array of biomedical topics.[1] These seminars have proved an ideal way to bring together clinicians, scientists, and others interested in contemporary medical history to share their memories. We are not seeking a consensus, but are providing the opportunity to hear an array of voices, many little known, of individuals who were 'there at the time' and thus able to question, ratify, or disagree with others' accounts – a form of open peer-review. The material records of the meeting also create archival sources for present and future use.

The History of Twentieth Century Medicine Group became a part of the Wellcome Trust's Centre for the History of Medicine at UCL in October 2000 and remained so until September 2010. It has been part of the School of History, Queen Mary University of London, since October 2010, as the History of Modern Biomedicine Research Group, which the Wellcome Trust

[1] See pages 111–117 for a full list of Witness Seminars held, details of the published volumes, and other related publications.

funds principally under a Strategic Award entitled 'The Makers of Modern Biomedicine'. The Witness Seminar format continues to be a major part of that programme, although now the subjects are largely focused on areas of strategic importance to the Wellcome Trust, including the neurosciences, clinical genetics, and medical technology.[2]

Once an appropriate topic has been agreed, usually after discussion with a specialist adviser, suitable participants are identified and invited. As the organization of the seminar progresses and the participants list is compiled, a flexible outline plan for the meeting is devised, with assistance from the meeting's designated chairman/moderator. Each participant is sent an attendance list and a copy of this programme before the meeting. Seminars last for about four hours; occasionally full-day meetings have been held. After each meeting the raw transcript is sent to every participant, each of whom is asked to check his or her own contribution and to provide brief biographical details for an appendix. The editors incorporate participants' minor corrections and turn the transcript into readable text, with footnotes, appendices, a glossary, and a bibliography. Extensive research and liaison with the participants is conducted to produce the final script, which is then sent to every contributor for approval and to assign copyright to the Wellcome Trust. Copies of the original, and edited, transcripts and additional correspondence generated by the editorial process are all deposited with the records of each meeting in the Wellcome Library, London (archival reference GC/253) and will be available for study.

For all our volumes, we hope that, even if the precise details of the more technical sections are not clear to the non-specialist, the sense and significance of the events will be understandable to all readers. Our aim is that the volumes inform those with a general interest in the history of modern medicine and medical science; provide historians with new insights, fresh material for study, and further themes for research; and emphasize to the participants that their own working lives are of proper and necessary concern to historians.

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[2] See our Group's website at www.histmodbiomed.org.

ACKNOWLEDGEMENTS

We are very grateful to Professor Jon Cohen, Professor Sir Mark Walport, and Professor Fran Balkwill for independently suggesting the topic for this seminar, and also for advising us on suitable participants and the intended programme for discussion. Many thanks also to Professor Fran Balkwill for chairing the meeting so successfully.

As with all our meetings, we depend a great deal on Wellcome Trust staff to ensure their smooth running: the Audiovisual Department, Catering, Reception, Security, and Wellcome Images. We are also grateful to Mr Akio Morishima for the design and production of this volume; the indexer Ms Cath Topliff; Mrs Sarah Beanland and Ms Fiona Plowman for proofreading; Mrs Debra Gee for transcribing the seminar; Ms Caroline Overy for assisting with running the seminar and Mr Adam Wilkinson who assisted in the organization and managing of the meeting. Finally, we thank the Wellcome Trust for supporting the Witness Seminar programme.

Tilli Tansey

Apostolos Zarros

School of History, Queen Mary University of London

ILLUSTRATIONS AND CREDITS*

* Unless otherwise stated, all photographs were taken by Mr Wilde Fry, Wellcome Trust, and reproduced courtesy of the Wellcome Library, London.

ABBREVIATIONS

ASS	argininosuccinate synthase
BCG	British Cytokine Group
CAR	chimeric antigen receptor
CAT	Cambridge Antibody Technology
CD4	cluster of differentiation 4
CD95	cluster of differentiation 95
cDNA	complementary DNA
CTLA4	cytotoxic T lymphocyte-associated protein 4
DKFZ	Deutsches Krebsforschungszentrum (German Cancer Research Center)
DMBA	7,12-dimethylbenz[a]-anthracene
EMA	European Medicines Agency
ERC	European Research Council
EULAR	European League Against Rheumatism
Fc	fragment crystallizable
FDA	Food and Drug Administration (USA)
GM-CSF	granulocyte-macrophage colony-stimulating factor
GSK	GlaxoSmithKline
HLA-DR	human leukocyte antigen – antigen D-related
ICRF	Imperial Cancer Research Fund (UK)
IFN	interferon
IFN-β	IFN beta
IFN-γ	IFN gamma
IgG	immunoglobulin G
IgM	immunoglobulin M

IL-1	interleukin-1
IL-1α	IL-1 alpha
IL-1β	IL-1 beta
IL1B	human gene for IL-1β
IL-2	interleukin-2
IL-6	interleukin-6
IL-8	interleukin-8
JNK	Jun kinase
LPS	lipopolysaccharide
MAPK	p38 mitogen-activated protein kinase
MIF	(macrophage) migration inhibition factor
MMPs	matrix metalloproteinases
MRC	Medical Research Council (UK)
mRNA	messenger RNA
MS	multiple sclerosis
MTAs	Material Transfer Agreements
NEJM	*New England Journal of Medicine*
NFκB	nuclear factor kappa-light-chain-enhancer of activated B cells
NHS	National Health Service (UK)
NIH	National Institutes of Health (USA)
PD1	programmed cell death protein 1
PI	principal investigator
PNAS	*Proceedings of the National Academy of Sciences*
PoP	Proof-of-Principle
QMUL	Queen Mary University of London
RA	rheumatoid arthritis
RECIST	Response Evaluation Criteria In Solid Tumors

RIP1	receptor interacting protein 1
SLE	systemic lupus erythematosus
TCR	T cell receptor
T_h1	T helper 1 (response)
T_h17	T helper 17 (cell)
TNF	tumour necrosis factor
TNFα	TNF alpha
TNFR2	TNF receptor type 2
TPA	12-O-tetradecanoyl-phorbol-13-acetate
TRAIL	TNF-related apoptosis-inducing ligand
UCL	University College London
UNR	National University of Rosario
WIHM	Wellcome Institute for the History of Medicine

INTRODUCTION

The idea of doing a Witness Seminar on tumour necrosis factor (TNF) emerged during a dinner at the Royal College of Physicians, at which I found myself sitting next to Tilli Tansey. The story of TNF can illustrate many of the most fascinating and inspiring ideas in science: serendipity, collaboration, tenacity, the value of 'blue skies' research, basic science being translated into entirely novel treatments for patients with a common and disabling disease. On more than one occasion I have found myself talking to medical students about the excitement of academic medicine as a career and I have turned to the story of TNF, in particular the development of anti-TNF, to illustrate my case. It seemed to me that the topic was 'a natural' for a Witness Seminar, and this volume is the result.

It was serendipity that led to my own interest in TNF. In the early 1980s I was a young and aspiring infectious diseases physician, working on endotoxin (lipopolysaccharide, LPS) and the pathogenesis of severe Gram-negative infections. For reasons that I do not now recall, I was invited to talk to a meeting of orthopaedic surgeons, and I arrived in good time and joined the audience. The speaker before me was Fran Balkwill, who has so ably chaired this Witness Seminar. She was speaking about a substance which I had never heard of, called 'osteoclast activating factor', but which turned out to be TNF. I became very excited about the work of Beutler and Cerami and the idea that TNF might be a key mediator of septic shock. Fran was enormously helpful and put me in touch with Walter Fiers, who very generously provided reagents, and I started a programme of work, supported by the Wellcome Trust, to take this further. We and others showed that anti-TNF could protect experimental animals from septic shock after an LPS challenge and from live *E. coli* infection. The stage seemed set for a potential significant new treatment for patients with this life-threatening condition. We were approached by Alistair Riddell, then working at Celltech, who wanted to test a monoclonal antibody in patients with sepsis, and that led to Andrew Exley (a clinical training fellow in my lab) and I publishing a letter in *The Lancet* in 1990 describing the first clinical use of anti-TNF in patients with sepsis.[1] As several speakers in the seminar point out, anti-TNF as a treatment for sepsis sadly proved to be ineffective, but not before tens of millions (probably hundreds of millions) of dollars had been spent on a series of large clinical trials which investigated a series of different anti-TNF molecules.[2] Although ultimately unsuccessful, the tale of anti-TNF in sepsis is a good illustration of how hard it can be to turn good basic science into a commercially successful therapy.

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[1] Exley *et al.* (1990).

[2] See, for example, Abraham *et al.* (1998), Cohen and Carlet; International Sepsis Trial Study Group (1996), Panacek *et al.* (2004), Reinhart *et al.* (1996) and Rice *et al.* (2006).

It is against that background that this seminar sets out the story of how Tiny Maini and Marc Feldmann and their collaborators pushed forward with anti-TNF, resulting in a truly remarkable and genuinely life-changing treatment for patients with rheumatoid arthritis (RA). What comes out very clearly from their account is that doing the science was actually in many ways not the main challenge. It certainly required the initial intellectual insights to see the possibilities, and there were the usual challenges of solving the technical problems and of obtaining sufficient funds to be able to carry out the work. But some of the most difficult hurdles were in overcoming the practical and regulatory hurdles involved in carrying out clinical trials with a reagent (a monoclonal antibody) which, at that time, was very unfamiliar territory indeed for both clinicians and regulators. Simply manufacturing enough of the material to be able to do the study was not straightforward, and there were (at the time, entirely reasonable) concerns about potential side-effects such as increasing the risk of opportunistic infection. It is to their enormous credit that these obstacles were overcome, and millions of patients are indebted to them for their work.

The transcript also illustrates, I think, the hugely important role of clinician scientists in bringing about breakthroughs such as this. From their first-hand accounts, it is abundantly clear that it was the combination of basic scientists working closely alongside academic clinicians, that provided the particular set of skills and expertise that could bring an original scientific idea through the whole development process from bench to bedside. In today's National Health Service, with its highly structured and rather inflexible training programmes, and an intensely competitive academic environment that sets great store on basic science, it frequently seems to be the case that bright young clinicians must increasingly choose between a career in the research laboratory or training as clinical researchers. The inspiring story of anti-TNF for RA should serve as a model for young doctors and is one of the most powerful arguments for us protecting the species of the clinician scientist.

Professor Jon Cohen

Brighton & Sussex Medical School

Figure A

THE RECENT HISTORY OF TUMOUR NECROSIS FACTOR (TNF)

The transcript of a Witness Seminar held by the History of Modern Biomedicine Research Group, Queen Mary University of London, on 14 July 2015

Edited by A Zarros, E M Jones, and E M Tansey

THE RECENT HISTORY OF TUMOUR NECROSIS FACTOR (TNF)

Participants*

Professor Fran Balkwill (Chair)
Professor Yuti Chernajovsky
Professor Sir Marc Feldmann
Professor Joachim Kalden
Professor Sir Ravinder Nath (Tiny) Maini
Professor Jeremy Saklatvala
Professor Tilli Tansey
Dr Marcos Vidal†
Professor Henning Walczak
Assoc. Professor Richard Williams
Dr James (Jim) Woody (via phone from the USA)

† Died 2 January 2016

Apologies include: Professor Jon Cohen, Professor Andrew Cope, Professor Jeremy Farrar, Professor Walter Fiers, Professor David Goeddel, Professor Stephen Holgate, Professor David Isenberg, Professor Mike Owen, Dr Michael Shepard, Professor Josef Smolen, Professor Peter Taylor, Professor Sander Van Deventer, Dr Carrie Wagner, Professor David Wallach, Professor Sir Mark Walport, Sir Greg Winter

* Biographical notes on the participants are located at the end of the volume

Figures 1 and 2: Professor Fran Balkwill and Professor Tilli Tansey

Professor Tilli Tansey: Good afternoon everyone. I'm Tilli Tansey, the convenor of the History of Modern Biomedicine Research Group.[1] The purpose of these meetings is to get behind the hidden record, get behind the published literature, find out what really did happen: what were the key events, who were the key people – they may not be the key papers that we all know about. This meeting has been planned three times, and, as we were organizing it, very gradually the names on the top of your list of the confirmed attendees, migrated down to the bottom to apologies received as people found they were unable to come. And at one point we did think we would have to cancel, but, having tried three times to hold this meeting, we decided to continue.

I've had a lot of enthusiastic support from Jon Cohen and Mark Walport, and also from Fran Balkwill, to hold this meeting so we are going ahead although we are a little depleted today.[2] That said, tumour necrosis factor (TNF) is such an

[1] The History of Modern Biomedicine Research Group's website homepage is: http://www.histmodbiomed.org (accessed 11 March 2016).

[2] Professor Jonathan (Jon) Cohen, who has written the Introduction to the current Witness Seminar transcript, is Emeritus Professor at Brighton & Sussex Medical School (Universities of Brighton and Sussex), President of the International Society of Infectious Diseases, Member of the Scientific Advisory Board of the Lister Foundation, and a Non-Executive Director of King's National Health Service (NHS) Foundation Trust; http://www.arthritisresearchuk.org/about-us/how-we-are-managed/our-trustees/jon-cohen.aspx (accessed 11 March 2016); for more details, see biography on page 84. Professor Sir Mark Walport is the Government Chief Scientific Adviser and Head of the Government Office for Science; https://www.gov.uk/government/people/mark-walport#biography (accessed 11 March 2016).

important issue, such an important discovery, and the impact and possibilities of TNF are so important we did feel that we should hold a meeting and try to produce a volume as soon as we could. An important part of any meeting is identifying a suitable chairman: I'm not sure the word 'chairman' is right, it's more 'facilitator' to try and guide people through general memories and discussions. There's no formal agenda for this meeting, but we have put together an outline programme.[3] We want people to contribute informally as and when they wish to do so.

And so, without further ado, I'd like to introduce Fran, our chairman; our facilitator. Fran needs very little introduction; a very distinguished cancer researcher herself, she has been very keen to go ahead with this meeting. She is here as a facilitator but also, to a large extent, as a witness and participant herself. So Fran, over to you.

Professor Fran Balkwill: Thanks, Tilli, and thanks everyone for coming. It's just so great to finally be doing this. This has, as Tilli said, been an idea for a long time. My own TNF journey began, I'm not entirely sure whether it was 1984 or 1985, when I was sitting at my desk at the Imperial Cancer Research Fund (ICRF), Lincoln's Inn Fields, and the phone rang, and somebody I didn't know with a Belgian accent said, 'Hello, this is Walter Fiers.[4] I've been reading your papers on interferon (IFN). Would you like to work on TNF?' I had a gut feeling he was somebody terribly important so I said, 'Ooh yes, that sounds interesting.' I rushed up to the library, because I had no idea what TNF was, and, ploughing through *Index Medicus*, I got terribly excited. It really started a career-long involvement with a molecule that is involved in so many different areas of biology, and so this Witness Seminar can be about inflammation, inflammatory disease, but also about cancer cells, cell death, and many other things as well.

It's not my job to steer or say what should happen, so I would just finish this introduction by saying TNF is involved in everything, and I was reminded of this just last week when we had our tenth anniversary showcase at Barts Cancer Institute. Although I hadn't mentioned TNF very much in my talk, one of my ex-clinical fellows, who is now a principal investigator (PI), gave a talk where he reminisced quite warmly about his time in my lab doing his PhD.[5] During

[3] A draft outline programme was circulated to seminar participants to comment on a month in advance of this meeting. Table 1 is the final version of that programme used as a framework for this seminar.

[4] For Professor Walter Fiers, see biography on page 86.

[5] Dr Peter Szlosarek, Clinical Senior Lecturer at the Barts Cancer Institute.

Historical context and timeline of events
I Before 1970s: • State of play of rheumatoid arthritis (RA): diagnosis, treatment, and research • Understanding of autoimmune diseases
II 1970s – mid 1980s: • Understanding of autoimmune diseases • Role of cytokines in inflammation, immunity, and cell growth
III Mid 1980s – early 1990s: • Collaboration of Feldmann and Maini to study disease mechanism in RA • Identification of tumour necrosis factor (TNF) in diseased joints • Recognition of a potential therapeutic approach • Experimental, clinical, and commercial collaborations
IV Early 1990s – *c.*2000: • First clinical trials were performed (1992); infliximab from Centocor • Other companies focusing on TNF-related therapeutics • Applications for other diseases
V 2000s: • State of play of RA: diagnosis, treatment, and research • Understanding of autoimmune diseases
Areas of focus: • The role of funding and institutional support • Relations and collaborations between different groups of workers, including lab researchers, clinicians, industrial, and funding bodies • The acceptance and development of new theories • Translational research from the lab to the clinic, and then to commercial production

Table 1: Witness Seminar outline programme

it he'd discovered a molecule called 'argininosuccinate synthase' – it's known as 'ASS1' now – but at the time we all called it 'ASS', and we would tease the hell out of him. We said, 'You don't want to study that boring metabolic gene.' But, in fact, the talk he gave, this total persistence over the last 15 years, where he found it was a major TNF-related gene but in other cancer types, again showing this 'yin and yang' of TNF; it's actually a tumour suppressant. When you've got an absence of ASS in the tumour, you can deplete arginine and it actually is quite lethal for the cancer. This has now gone with worldwide clinical trials of this inhibitory pathway in diseases like mesothelioma. The reason I'm telling

this story is not just the fact that TNF is involved in so many areas of biomedical science, but he reminded me of how he ended up in my lab. Back in 1988 when Peter Szlosarek was doing his A levels, he was in the school library and he started reading *Scientific American* and he came across an article from Lloyd Old on TNF.[6] Tilli and I now both have a copy of this; you may like to look at it later. And it was that, and then doing his medical degree, hearing a talk at the ICRF that I gave apparently when he was doing a BSc, and then finding an advert in the *British Medical Journal* for a clinical fellowship working on TNF, that ended up all these years later with this story on a drug that targets cancer metabolism.

It was just really nice that because both Tilli and I could go back and find that Lloyd Old paper from 1988, so that really sums up TNF for me. I'm just really happy everyone is here and I look forward to an interesting afternoon, starting with thinking about the pre-1970s. Marc, maybe you would like to start?

Professor Sir Marc Feldmann: If we are discussing the state of play of rheumatoid arthritis (RA) diagnosis, treatment, research pre-1970s, I will do what I've often done and pass the microphone to my colleague, Tiny. [Laughter]

Professor Sir Ravinder Nath (Tiny) Maini: Okay, well, so that is really forcing the focus on RA.

Balkwill: To begin with, I think that's fine.

Maini: I'm a clinician but also a laboratory scientist, and I began training as a rheumatologist in 1966, when I joined a group of that small speciality and realized that as an undergraduate I wasn't taught very well about the scope of rheumatic diseases, and that RA was a very interesting and important disease. In the 1970s, it really was inadequately treated because there was no known significant therapy for it, and the therapeutics that were in the clinic had got there more or less by anecdotal evidence. A number of these drugs were routinely used by some rheumatologists, but not by others, and rheumatologists by and large were very strongly brought up on the ethics of Hippocrates 'do no harm to your patient'.[7] And so the instruction I received when I started treating RA

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[6] Old (1988). Lloyd John Old (1933–2011) was a leading figure in the field of tumour immunology. From 1971 to 2011, he served as the Scientific and Medical Director of the Cancer Research Institute (New York); http://www.cancerresearch.org/about/lloyd-j-old (accessed 11 March 2016).

[7] The phrase is indirectly contained in the Hippocratic Oath and the Hippocratic Corpus; in the latter the phrase 'ἀσκέειν, περὶ τὰ νουσήματα, δύο, ὠφελέειν, ἢ μὴ βλάπτειν', which can be translated as 'strive, with regard to diseases, for two (things), (namely) to do good or to do no harm'.

Figure 3: Professor Sir Ravinder Nath (Tiny) Maini

was to be very cautious with introducing drugs such as gold injections, which were used for the treatment of RA, their efficacy having been demonstrated in a controlled clinical trial in 1961.[8] However, gold was very toxic and it was known to cause severe rashes and bone marrow aplasia, renal damage, and so on.[9] Consequently, its use was, in some quarters, pretty well neglected.

The big breakthrough, of course, had come with the discovery of cortisone by Hench and others in 1948, and what he had demonstrated was a remarkable effect of cortisone on patients with RA.[10] And, interestingly, the clinic where I was attached as senior registrar in West London, was one of the first clinics to use cortisone treatment in the UK. And they had gone through a cycle of using cortisone and adrenocorticotropic hormone, quite extensively. They had a lot of experience with it, and had come to the conclusion that it was a pretty toxic

[8] Research Sub-Committee of the Empire Rheumatism Council (1961); see also note 11.

[9] See, for example, Lawrence (1953) and Bogg (1958).

[10] See, for example, Hench *et al*. (1949). Philip Showalter Hench (1896–1965) was awarded the Nobel Prize in Physiology or Medicine in 1950, with Edward Calvin Kendall and Tadeus Reichstein 'for their discoveries relating to the hormones of the adrenal cortex, their structure and biological effects'; http://www.nobelprize.org/nobel_prizes/medicine/laureates/1950/ (accessed 14 March 2016).

drug although it was very beneficial. The Empire Rheumatism Council[11] had conducted trials with cortisone and shown it to be effective, but its side-effects were a severe limitation, largely because it was overused.[12] So it's very interesting to reflect that, today, synthetic prednisone or its active metabolite, prednisolone, are both extensively used by rheumatologists in treating RA in lower doses or for induction therapy in higher doses for a very short time. Undoubtedly, it has a very important place in initially controlling disease activity and symptoms of RA.

In the 1970s there was essentially a failure in treating RA. It was commonplace in our outpatient clinics to see patients in wheelchairs, and half the clinic would be occupied by patients in wheelchairs. In those days we had a ward dedicated to the inpatient treatment of RA. Well, today, if you go to a rheumatology clinic, you won't see many wheelchairs. The rheumatology ward that we had at this hospital and, subsequently, at Charing Cross Hospital, disappeared. All patients are now treated as an outpatient and the quality of life they enjoy is hugely improved, and we can come back to that in a minute.

Let me begin with a very small picture of the evolution of the treatment of RA by stating that the major clinical trials for treating RA occurred only in the 1980s, and the first drug that impressed rheumatologists for its efficacy in a short-term clinical trial was methotrexate.[13] Methotrexate had been used previously in the 1950s,[14] but again anecdotally and in doses which were thought to be not toxic; in other words there were very low doses used intermittently, weekly, compared to the doses that we use for treating cancer. Interesting that there is a crossover between cancer and RA with this drug treatment, as there is with other anti-rheumatoid drugs.

And, of course, other cytotoxic drugs were also used empirically, like azathioprine, chlorambucil, and cyclophosphamide.[15] All of these drugs were used because of their potential at inhibiting cell division, which inhibited the immune response

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[11] The Empire Rheumatism Council was founded in 1936. In 1964 it became the 'Arthritis and Rheumatism Council', while in 1998 it was re-named the 'Arthritis Research Campaign'. In 2010 the charity became 'Arthritis Research UK'. For more details, see http://www.arthritisresearchuk.org/arthritis-information/arthritis-today-magazine/151-winter-2011/back-to-the-future.aspx (accessed 16 March 2016).

[12] See, for example, Research Sub-Committee of the Empire Rheumatism Council (1957).

[13] See, for example, Ward (1985) and Willkens (1985).

[14] For methotrexate, see Sir Kenneth Calman's 'praise poem' in volume 30 of the Wellcome Witnesses to Twentieth Century Medicine series: Christie and Tansey (2007), pp. 78–81.

[15] See, for example, Luqmani, Palmer, and Bacon (1990).

as well. But the well-controlled clinical trials really weren't done till the 1980s, when other effective drugs emerged, and included not only methotrexate, but a drug called 'salazopyrin', also known as 'sulphasalazine', and gold.[16] So those were the essential drugs that were implemented in practice in the 1980s.

Professor Yuti Chernajovsky: At the time that you just described, what was the hypothesis regarding the mechanism of RA? Were people talking of autoimmunity at that time already?

Maini: Yes, I think that the discovery of rheumatoid factor was described in 1940 by Erik Waaler in Norway.[17] Subsequently a test was developed, called the 'Rose–Waaler test', which was routinely used by rheumatologists, and which is a test for immunoglobulin M (IgM) class of the rheumatoid factor; an autoantibody directed against determinants in the fragment crystallizable (Fc) portion of immunoglobulin G (IgG).[18] So RA had joined systemic lupus erythematosus (SLE) as an autoimmune disease based on this diagnostic test. In the 1960s there was quite a lot of interest in the microscopic histology of the joint in RA, and there were seminal papers from many groups, including that of Morris Ziff in Texas, also looking at ultrastructural level in the synovium, and describing a heavy infiltration with immune cells, predominantly lymphocytes, some of them arranged in aggregates or follicles.[19]

Balkwill: Would they be like what we call 'tertiary lymphoid structures' now, do you think?

Maini: Two kinds of infiltration were observed: firstly, diffuse infiltration, and secondly, a more organized structure. The organized structure tends to occur in more chronic disease, but all patients also have massive diffuse lymphocyte infiltration, predominantly by T cells of certain subtypes, and by B cells, and, of course, by monocytes.

Balkwill: So if we stay on the RA theme for a minute, when did the first ideas arise about TNF being involved in RA?

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[16] For the use of salazopyrin in the treatment of RA, see Pullar, Hunter, and Capell (1987).

[17] Erik Waaler (1903–1997) was a Norwegian Professor of Pathology at the University of Bergen; for the original article describing the discovery of the rheumatoid factor, see Waaler (1940).

[18] The Waaler–Rose test is a serotological test for diagnosing RA that has been based upon the Waaler's 1940 test, and was (also) elucidated by Rose *et al.* (1948), without prior knowledge of his discovery; for a more detailed account, see Alexander (1967–1968).

[19] See, for example, Ziff (1965).

Figure 4: Professor Joachim Kalden

Professor Joachim Kalden: I was Head of the Department of Internal Medicine that specialized in clinical immunology, rheumatology, and haematology at the Institute for Clinical Immunology at the University of Erlangen-Nürnberg. When I finished my medical studies in Germany in 1965, I had rarely seen a patient suffering from any type of rheumatoid disease. The textbooks were nearly blank. Rheumatic diseases were treated at this time in spa areas. The major medication included gold injection and physiotherapy. Interest in a more intensive study in the pathogenesis and the development of new treatment principles of rheumatic diseases started at the end of the 1960s/1970s, when quite a number of young German academic fellows with an interest in autoimmune diseases returned from a long stay abroad. I, for example, spent a couple of years at the University of Edinburgh studying autoimmune diseases of the neuro- and endocrine systems. With regard to rheumatic diseases, studies centred on SLE and, later on, also on RA. By the end of the 1980s, based on studies into the pathogenesis of RA, we developed mouse monoclonal antibodies targeting the CD4 molecule of T cells;[20] the results we obtained in an open label trial with a mouse monoclonal antibody were very promising, but later on in placebo-controlled trials, it became quite clear that targeting the CD4 molecule was not the way for an effective intervention into the course of RA. With these negative

[20] CD4: cluster of differentiation 4; a glycoprotein found on the surface of immune cells, helping them to communicate with an antigen-presenting cell.

data, T cells as targets disappeared for a while until a new biologic, abatacept, which blocks the interaction between dendritic cells and T cells in RA, was nearly as efficacious as blocking TNF alpha (TNFα).

Feldmann: The work that Tiny and I did began by focusing on cytokines. This came about by thinking why was it that most autoimmune disease sites, which were infiltrated by lymphocytes and macrophages, had dramatic upregulation of human leukocyte antigen – antigen D-related (HLA-DR) molecules. In the 1970s, the dominant theme for autoimmunity was its human leukocyte antigen (HLA) region linkage, genetically.[21] It was found that, essentially, all the autoimmune diseases were linked to HLA class II, some of them a little bit strangely. SLE was much more a complement linkage – some complement genes are in the middle of the HLA region – than actually HLA itself. Autoimmunity, that was the key driver for thyroid disease, diabetes, RA, and so on. My involvement in autoimmunity dates back to my PhD studies.

In the modern era, autoimmunity was initially a theoretical construct, proposed first by Paul Ehrlich,[22] but defined by the work of Sir Frank Macfarlane Burnet;[23] he had been the director of the Walter and Eliza Hall Institute,[24] where I did a PhD, and so this was something that I was always interested in. Then, when I was in London studying immune regulation, the role of HLA-DR molecules in presenting antigen, Gian Franco Bottazzo and Deborah Doniach came to visit me.[25] They were at the Middlesex Hospital in London, I was at University College London (UCL); not part of one institution at the time but about 500 yards apart. They showed me histological pictures of thyroid disease and diabetes with dramatically upregulated HLA-DR and were very excited about how useful this might be as a marker. They asked me what did I think this might mean. Actually, if you are an immunologist, then of course HLA-DR is more than a marker – it has a function – it's important in antigen presentation, so my response was fairly clear. This upregulated HLA-DR suggested that antigen presentation is locally important, and this discussion, which took place

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[21] See, for example, Bodmer (1980) and Gough and Simmonds (2007).

[22] For more details on the development of the concept of 'autoimmunity', see Silverstein (2001).

[23] See, for example, Burnet (1961).

[24] The Walter and Eliza Hall Institute (founded in 1915) is an Australian medical research institute; see a brief history at http://www.wehi.edu.au/about/history (accessed 21 March 2016).

[25] Professor Gian Franco Bottazzo (b. 1946) was the first researcher to recognize that type 1 diabetes was an autoimmune disease. For Deborah Doniach (1912–2004), see Tansey *et al.* (1997) and obituary by Wright (2013).

probably in late 1982, ended up as a joint publication. It was a hypothesis paper in *The Lancet* with Franco as the first author, but actually I wrote it all and I was the last author.[26] Basically, what it pointed out was that if antigen presentation was upregulated in a local tissue site which naturally expressed autoantigens, then because tolerance to self antigens is not locked in, it's not a deletional tolerance, it's a form of regulation; hence there was an opportunity for triggering immune responses. So we published this hypothesis paper in 1983, discussing upregulation of HLA-DR, upregulation of antigen presentation, and the pathogenesis of autoimmunity.

That encounter had really started me thinking about autoimmunity, and the paper was the primer to try to test that hypothesis. The hypothesis could be tested at the cellular level in thyroid disease. I was working with Bottazzo, and since thyroid disease is classically treated by surgery, we used autoimmune hyperthyroidism specimens as a model in which we got a lot of lymphocytes and a lot of epithelial thyroid tissue. We showed, with the help of Jonathan Lamb, a postdoc, that T cell clones that recognized peptides could be restimulated both by classical, antigen-presenting cells, but also by epithelial cells which expressed HLA-DR and had been soaked with the relevant peptides,[27] and, subsequently with Marco Londei, that the autoimmune disease had autoantigen reactive T cells that recognized the endogenous peptides.[28]

So from this 1982 discussion came a hypothesis that was, I think, quite influential at the time. It led to a whole plethora of transgenic mice producing various cytokines locally, and produced by several groups: Nora Sarvetnick was the first one. She put IFN gamma (IFN-γ) genes under control of the insulin promoter, and this generated autoimmune diabetes as local production of the cytokine drove up antigen presentation.[29]

Balkwill: Ah, that's the link.

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[26] Bottazzo *et al*. (1983).

[27] Londei *et al*. (1984). Professor Jonathan Lamb is now Chair of Immunoregulation in the Division of Cell and Molecular Biology, Imperial College London; http://www.imperial.ac.uk/people/jonathan.lamb (accessed 21 March 2016).

[28] Londei, Bottazzo, and Feldmann (1985).

[29] Sarvetnick *et al*. (1988). Professor Nora E. Sarvetnick is Director of the Holland Regenerative Medicine Program and Professor at the Department of Surgery of the University of Nebraska; http://www.unmc.edu/surgery/divisions/transplant/faculty/sarvetnick.html (accessed 21 March 2016).

Figure 5: Professor Sir Marc Feldmann

Feldmann: The over-produced cytokine could be shown to induce autoimmunity. My path went in a different direction: once I knew that the cellular immunology in a human autoimmune disease was consistent with this hypothesis, my interest was to go back to my medical roots and do something therapeutically important, and that couldn't be done in thyroid disease. The treatment of thyroid disease is very simple with very effective and very cheap surgery, and therefore the next issue was which autoimmune disease to look at.

At UCL, in Avrion Mitchison's group, there had been a visiting rheumatologist, Nathan Zvaifler, who came to spend a sabbatical with him, but ended up in my tender, loving care.[30] I taught him a little bit of immunology and he taught me a little bit of rheumatology, so when I thought RA might be a useful disease model to continue with this study, to try and work out what mediators might be important, I asked him who I should contact. He told me exactly the person: Ravinder Maini, two or three miles down the road. I contacted Tiny, and that's essentially how autoimmune mechanistic ideas on HLA regulation linked to cytokines moved from infertile ground in thyroiditis to what became a very fertile area: RA.

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[30] Professor (Nicholas) Avrion Mitchison (b. 1928) is Professor Emeritus and has served as Professor of Zoology and Comparative Anatomy at UCL. Nathan Zvaifler (1927–2015) was a Professor of Rheumatology at the San Diego School of Medicine, University of California.

Maini: I just want to pick up a point that Marc is making about HLA presentation and the importance of T cells. I think that our thinking in the 1980s became very strongly entrenched in the model that Marc has outlined, that basically all autoimmune disease would turn out to be HLA-dependent antigen-presenting diseases. Now the problem in RA was that there wasn't a well-characterized autoantigen, apart from Ig as the antigen for rheumatoid factor, and rheumatoid factors, of course, occur in many diseases, such as chronic infections or chronic liver disease and are not rheumatoid-specific – any chronic inflammatory disease will be accompanied by rheumatoid factor. So there was serious doubt that rheumatoid factor was pathogenic, unlike DNA and DNA antibodies, which were thought to be *the* pathogenic molecules in SLE.

Balkwill: And that was another reason why RA was a good choice as a model?

Maini: Yes, indeed. I think Marc will recall that we spent quite a lot of time in that initial period of collaboration on studying T cells. In fact, Marco Londei, working in Marc's laboratory, did isolate T cell clones from a rheumatoid joint reactive with collagen type II.[31] Now, collagen type II was a candidate autoantigen for RA proposed by Swedish workers,[32] as it is expressed predominantly in the cartilage of joints in humans, and could have been the key autoantigen driving the immune response. Today, we know that it is only one of the important autoantigens, not as 'native' collagen but as post-translated citrullinated collagen antigen, in a proportion of rheumatoid patients. So today we accept the dogma that citrullinated proteins that are expressed at sites of inflammation – amongst which the best described ones are citrullinated forms of enolase, vimentin, and fibrinogen – are essentially the predominant targets of the immune response that give rise not only to corresponding specific T cells, but also to specific IgG antibodies.

When I first started to take an interest in the pathogenesis of RA, there were two major findings that were of interest. One was that immune complexes were present in the joint,[33] and these were characterized as 'IgG rich', but it was not possible to identify what the antigen component of these complexes was, other than that rheumatoid factors self-associated. The second was that patients with RA had depressed, delayed-type sensitivity reactions to skin tests. At that time

[31] Londei *et al.* (1989). Dr Marco Londei is Chief Development Officer at AnaptysBio; http://www.anaptysbio.com/marco-londei/ (accessed 21 March 2016).

[32] Klareskog *et al.* (1983).

[33] For example, see Bourke *et al.* (1982).

it was becoming evident from the work of Barry Bloom and John David that delayed-type sensitivity was mediated by T cells and soluble factors such as the macrophage migration inhibition factor (MIF).[34]

Balkwill: And presumably things like TNF as well?

Maini: Well, we didn't know about TNF at that time but had been informed about a cytotoxic factor called 'lymphotoxin'. I think Jeremy Saklatvala might pick that story up about TNF. But MIF was the predominant, soluble factor that was described in 1966, I think, Marc, wasn't it?[35] Anyway, MIF was the hypothetical soluble mediator of a delayed hypersensitivity reaction that might be implicated in the pathogenesis of inflammation observed in RA. But, paradoxically, patients with RA were deficient in delayed-type sensitivity skin reactions, so there was a competing concept that it may be an immune-deficient disease.

My fellowship at the bench was undertaken with an immunologist, Dudley Dumonde, who was one of the pioneers of the cytokine field in London.[36] He was at St Mary's and subsequently at the Kennedy Institute of Rheumatology.[37]

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[34] Bloom and Bennett (1966) and David (1966). Professor Barry R. Bloom is Harvard University Distinguished Service Professor and Joan L. and Julius H. Jacobson Professor of Public Health at the Harvard T. H. Chan School of Public Health; http://www.hsph.harvard.edu/barry-bloom/ (accessed 21 March 2016). Professor John David is Emeritus Richard Pearson Strong Professor of Tropical Public Health at the Harvard T. H. Chan School of Public Health; http://www.hsph.harvard.edu/john-david/ (accessed 21 March 2016).

[35] Bloom and Bennett (1966).

[36] The late Dudley Dumonde was Head of the Immunology Division of the Kennedy Institute from the late 1960s to 1977. He pioneered work on soluble factors generated by activated lymphocytes, which acted as 'messenger molecules' mediating a wide variety of biological activities on cells in their local environment. He coined the term 'lymphokines' to describe these heterogeneous activities, which in the 1980s became characterized as several molecules belonging to the 'cytokine' family.

[37] The Kennedy Research Institute of Rheumatology was an independent research centre managed by The Mathilda and Terence Kennedy Institute of Rheumatology Trust (now 'The Kennedy Trust for Rheumatology Research') from its foundation in 1996 to 2000. In 2000, the scientific staff of the Research Institute were incorporated as a Division in the Faculty of Medicine at Imperial College. In 2011, the Kennedy Research Institute of Rheumatology transferred to Oxford University as an autonomous research centre within the Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences; for more details, see http://www.kennedy.ox.ac.uk/about/our-history (accessed 10 June 2016). At Oxford, the Kennedy Research Institute of Rheumatology received a substantial grant from the Kennedy Trust for a new building, equipment, and staff, and continues to receive financial support originating from royalty income on Feldmann and Maini patents on anti-TNF therapy owned by the Trust.

Figure 6: Associate Professor Richard Williams

I actually joined his lab at St Mary's in London and we worked with soluble mediators, and in 1969, with Dudley Dumonde, I published a paper on a factor called 'lymphocyte mitogenic factor' in *Nature*.[38] He simultaneously published a paper on lymphocyte mitogenic factor in mice, which he termed a 'lymphokine', and this factor also had other activities; in that way, lymphocyte-derived factors became known as 'lymphokines'.[39] But soon thereafter, other people working with monocytes described similar factors that monocytes were producing, so they became known as 'monokines', and some of these had chemotactic activity and they were called 'chemokines'. Then, I believe, at an international nomenclature meeting, which I'm sure Marc was probably attending, it was decided that we should lump all these soluble factors together and call them all 'cytokines'.

Associate Professor Richard Williams: I wanted to expand a little bit on what Tiny mentioned. I joined the Kennedy Institute in 1989, and prior to then I'd been working on an animal model of RA in which you take genetically susceptible mice and immunize them with collagen type II, which is the major constituent of cartilage, and they develop an RA-like disease.[40]

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[38] Maini *et al.* (1969).

[39] Dumonde *et al.* (1969).

[40] Courtenay *et al.* (1980), Williams and Whyte (1989), and Williams, Whyte, and Waldmann (1989).

So we had the animal model, coupled with the description of antibodies to collagen type II in the sera of patients with RA, as well as the studies by Marco Londei in Marc's lab, who had cloned T cells specific for collagen type II. There was this very strong hypothesis that RA was in fact a form of collagen-induced arthritis.

I joined the Institute at this stage under the supervision of David Williams[41] and our role was, in the first place, to look for and identify patients with antibodies to collagen type II. As for the results, we identified a small handful of patients with antibodies to native collagen type II, so basically the hypothesis was not founded.

Kalden: As I mentioned before, back in the 1970s and early 1980s, we were interested in T cells as a target for immune intervention in RA. The interest was based on data coming from patients and from *in vitro* experiments, as well as from experimental animal models. Thus, Frank Emmrich in the Institute developed a mouse anti-CD4 monoclonal antibody.[42] We did a Phase 1 trial, the data was perfect, so we thought we had it.[43] Later on, placebo-controlled trials demonstrated that targeting CD4 was not the molecule to lead to a significant improvement of signs and symptoms.[44] But again, as indicated before, after the T cells disappeared as a target in RA, they came back with a fusion protein which blocks the interaction; something that would lead to a significant improvement in the clinical course of RA patients.

Balkwill: I think it's quite good to carry on on the rheumatoid theme for a bit, and then come back to the cancer story. My memory from being interested in TNF in the cancer field was that then there were some papers where over-expression of TNF caused RA in mice. So how did the field move from T cells to anti-TNF?

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[41] Dr David (Gareth) Williams was an ARC Senior Research Fellow (1983–1996) at the Kennedy Institute of Rheumatology.

[42] Emmrich *et al.* (1991). Professor Kalden noted: 'when this monoclonal antibody was used in an open type Phase 1 trial, 40 to 50% of patients showed an improvement in signs and symptoms with a depletion of CD4+ T cells.' E-mail to Dr Apostolos Zarros, 5 April 2016. For more details, see Horneff *et al.* (1991). Professor Frank Emmrich is Chairman of the Department of Clinical Immunology and Director of the Institute of Clinical Immunology at the University of Leipzig; see http://www.ethikrat.org/about-us/members/frank-emmrich (accessed 21 March 2016).

[43] Horneff *et al.* (1991).

[44] Breedveld (1998) and van der Lubbe *et al.* (1993).

Figure 7: Professor Jeremy Saklatvala

Professor Jeremy Saklatvala: In the 1970s, people became aware that there were small proteins made by lymphocytes, monocytes, and macrophages that seemed to be hormones of inflammation and immunity. Tiny has mentioned their collective terms: 'lymphokines' and 'monokines' – lumped together they were called 'cytokines'. I was one of a number of people who were trying to isolate these mysterious proteins. Were there two or three of them, or a hundred of them, or what? I was a rheumatologist by training, but had moved into basic research because I was interested in biochemical mechanisms of tissue destruction in inflammation, which was the major clinical problem in RA and other similar inflammatory diseases. I was working at a lab in Cambridge called the 'Strangeways Research Laboratory' with Dame Honor Fell, John Dingle, Alan Barrett, and John Reynolds.[45] They were among the first people to define the proteolytic enzymes that controlled the turnover of the proteins that formed the extracellular matrix of connective tissues; these were the collagens and proteoglycans.

I joined the Strangeways lab in 1976 when Honor Fell had just shown an interesting activity that the joint lining tissue – the synovial membrane – expressed in culture.[46] This was due to a substance that stimulated cartilage

[45] For more details on the Strangeways Research Laboratory (founded in 1905, Cambridge) and the research work undertaken at the time, see Dingle (1979).

[46] Fell *et al.* (1976).

to reabsorb by causing the chondrocytes to dissolve their extracellular matrix. Subsequently, we found it had a similar effect on bone. We thought this could be a mediator made in inflammation, so a big effort was made to purify it. The active protein came to be called 'interleukin-1' (IL-1), though at that time we had given it another name, 'catabolin', because it caused catabolism of the extracellular matrix. That was one of the strands of research leading to the defining of various biological activities of IL-1.

Historically, the oldest of these activities was that of 'endogenous pyrogen', a substance from leukocytes that caused fever. Paul Beeson in 1948 was the first person to identify this.[47] But protein purification techniques were not good enough to purify these very low abundance proteins, which worked at very low concentrations, until the late 1970s and early 1980s. Molecular cloning didn't really become practicable until the early-to-mid 1980s. Therefore, there was a long wait until these molecules were obtained in useful amounts. So our protein that was causing the tissue resorption was a molecule that had come to have several names, 'endogenous pyrogen' being the first. That became 'IL-1'.

Initially, there was confusion because two different molecules, having identical biological properties, had actually been isolated. One became 'IL-1 alpha' (IL-1α), and the other became 'IL-1 beta' (IL-1β). They were only about 20 per cent homologous in their amino acid sequence, but they did have a common receptor so that explained their common properties. So we were all very excited about IL-1 as a driver of tissue resorption, and as a very potent pyrogen. We had isolated a molecule that caused cartilage destruction when injected into a rabbit knee, but if you injected a few nanograms of it into a rabbit brain, the animal started shivering and its body temperature was rapidly elevated. And so we thought, 'Ah, this is really important stuff.' That takes us to about 1984/85.

In 1985, I was in Boston visiting Charles Dinarello, a fever physiologist and one of the pioneers of IL-1.[48] He told me a very interesting story. He asked if I had heard of Coley's toxin and TNF: Coley had claimed that some microbial toxins caused regression of cancers.[49] Later investigators had defined a substance that caused tumour cell necrosis in serum of animals injected with bacteria.

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[47] Beeson (1948).

[48] For Professor Charles Dinarello, see biography on page 84.

[49] See, for example, Coley (1893). A useful short account of Coley's mixed toxins is provided by Balkwill (2009). Moreover, a timeline of important events in the history of TNF is provided in Appendix 1.

Charles told me that Genentech,[50] among other biotechnology companies, had prepared this cytokine in large quantities to try as a cancer treatment. However, in a Phase 1 clinical trial in patients with cancer, the recombinant TNF had caused high fever.[51]

Balkwill: I think one died, actually.

Saklatvala: Because the protein was made in bacteria, Genentech thought they might not have got rid of all the endotoxin and traces of this were causing fever, so they screened their preparations again for endotoxin. They thought they'd eliminated it, so why are these patients getting so sick? Charles was a leading fever physiologist and cytokinologist, so they sent the TNF to him, asking: 'Do you think this TNF is a pyrogen, or are there pyrogenic microbial contaminants in it?' So Charles did a number of experiments, mainly on mice, and came to the conclusion that TNF was a pyrogen in its own right, and similar to IL-1.[52] He gave this molecule to other people investigating various different activities of IL-1, asking: 'Does TNF have effects similar to IL-1? Is this tumour-killing molecule really another inflammatory hormone like IL-1?' Generally, we found that TNF did all the things that IL-1 would do, but you needed about ten times as much of it to cause the same effect.

Balkwill: Was it because you were using recombinant human TNF in the mice? Because I remember that human TNF wasn't as toxic to mice as mouse TNF.

Saklatvala: I don't know. I think it is a genuine difference, possibly due to difference in affinity for the receptors.

Another strand to the story, although I wasn't personally involved in this, was that TNF was also discovered as 'cachectin' by Bruce Beutler and Tony Cerami at the Rockefeller University.[53] Their protein was responsible for the general muscle wasting or cachexia of cancer. They did some experiments with Jean-

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[50] Genentech (founded in 1976) is a biotechnology company that is now a part of the Roche Group; see http://www.gene.com/media/company-information/chronology (accessed 21 March 2016).

[51] Creagan *et al.* (1988) and Selby *et al.* (1987).

[52] Dinarello *et al.* (1986).

[53] Beutler and Cerami (1986). Bruce A. Beutler (b. 1957) was awarded one half of the 2011 Nobel Prize in Physiology or Medicine, jointly with Jules A. Hoffmann, for 'their discoveries concerning the activation of innate immunity'; http://www.nobelprize.org/nobel_prizes/medicine/laureates/2011/press.html (accessed 15 March 2016). Anthony (Tony) Cerami (b. 1940) is the founder and Chairman of the Board of Directors of Araim Pharmaceuticals; http://anthonycerami.org/site/ (accessed 15 March 2016).

Michel Dayer, who was a bit like us in that he was looking at production of proteinases by connective tissue cells but in a slightly different way.[54] They found that cachectin stimulated fibroblasts to make collagenase and prostaglandins. These experiments were all being done around the same time, and the upshot was really that TNF was an IL-1-like inflammatory hormone, but it was also cytotoxic to some tumour cells. A striking difference between them that was shown by Charles Dinarello and others in the paper in the *Journal of Experimental Medicine* in 1986, I think, where they showed TNF was a pyrogen, and that TNF could cause production of IL-1, but IL-1 didn't cause the production of TNF.[55] That's how TNF became an inflammatory cytokine, and really the cell death side of it has always been a bit of a mystery to me because to make it cytotoxic you have to do tricks in tissue culture. The original Lloyd Old paper in the *Proceedings of the National Academy of Sciences (PNAS)* has the famous pictures of the black necrotic transplanted tumours, and I think it is possible that the TNF was causing thrombosis of the precarious blood supply of these tumours.[56] It was possibly killing them by causing infarction. But I think the anti-cancer aspect never bore fruit, and what we had was an inflammatory mediator. Then people said, 'Are these potent inflammatory hormones present at sites of inflammation?', and I don't know who was really first to show this in, for example, RA. The Edinburgh group of George Nuki and Gordon Duff measured IL-1 and TNF in synovial fluid,[57] and Marc Feldmann and his colleagues were carrying out similar investigations.[58]

Balkwill: I think that we could go in two directions now: back to the cancer or on with the rheumatoid story. I think we should carry on with the rheumatoid a bit longer, but there are a few things I'm sure others would like to come back on; for example, cell death, especially in those early days.

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[54] Dayer, Beutler, and Cerami (1985). Jean-Michel Dayer is Emeritus Professor of Medicine at the University of Geneva; http://loop.frontiersin.org/people/295807/bio (accessed 22 March 2016).

[55] Dinarello *et al.* (1986). For Professor Charles Dinarello, see note 48.

[56] Carswell *et al.* (1975).

[57] See, for example, Waalen *et al.* (1986), Di Giovine *et al.* (1987) and Di Giovine, Nuki, and Duff (1988). Professor George Nuki is Emeritus Professor of Rheumatology in the University of Edinburgh; http://www.research.ed.ac.uk/portal/en/persons/george-nuki(287eed5a-9efc-47dd-9c6a-ebd2c377536b).html (accessed 22 March 2016). Professor Sir Gordon Duff is the Principal of St Hilda's College (University of Oxford); http://www.sthildas.ox.ac.uk/college/college-non-academic-staff (accessed 22 March 2016).

[58] Buchan *et al.* (1988).

Feldmann: I'd like to pick up on the very important bit of science, which was how did these potent supernatants end up as very defined, precise molecules? Instrumental in moving the field forward was Jo Oppenheim, who started to organize conferences on these mediators, from about 1978, first in Washington but then they became global, to try and get the people in the field together to discuss their findings.[59] I was privileged enough to be a friend of his and to go to all of these. And it's from these meetings, as they got bigger, that the nomenclature 'IL-1', 'interleukin-2 (IL-2)', 'cytokines', 'monokines', 'lymphokines', became much more adopted. But I think it's important to bring out that the molecular basis of this field was dominated by the biotech industry, which was in a very different form to what biotech is today, and companies like Immunex and Genentech and a few others cloned most of the inflammatory mediators.[60] The first one cloned in 1979 was IFN beta (IFN-β) by Tada Taniguchi,[61] but from then on it was essentially by the companies. As you know, for the definition of molecules in human disease tissue, the tools were really important. Without the appropriate tools, progress would not be made. So the cloning of cytokine genes, of the TNF family, was done at Genentech, of IL-1 was done in Immunex and, partly, by Charles Dinarello,[62] and we could spend the afternoon discussing some of the entertaining behaviour that went on with their competition, but we won't. [Laughter] And Hoffmann-LaRoche[63] was also involved in IL-1, but I think they didn't clone it as such; they did the biochemistry.

Saklatvala: They cloned the mouse one, which was actually a gene for IL-1α.

Feldmann: I'm talking about human diseases.

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[59] Dr Joost (Jo) J. Oppenheim is a Senior Investigator and the Head of the Cellular Immunology Section at the Center for Cancer Research of the National Cancer Institute at Frederick, MD, USA; https://ccr.cancer.gov/Cancer-and-Inflammation-Program/joost-j-oppenheim?qt-staff_profile_tabs=3#qt-staff_profile_tabs (accessed 16 March 2015). For more details on the conferences, see Feldmann (2009).

[60] Immunex (founded in 1981, Seattle) is a biotechnology corporation acquired by Amgen in 2002. Details of Immunex Corporation Records, 1983–2002, are available on Archives West; http://archiveswest.orbiscascade.org/ark:/80444/xv43105 (accessed 22 March 2016). For Genentech, see note 50.

[61] Taniguchi, Fujii-Kuriyama, and Muramatsu (1980). Professor Tadatsugu Taniguchi is Emeritus Professor at the University of Tokyo; http://www.iis.u-tokyo.ac.jp/~mol-immu/en/top/biography.html (accessed 22 March 2016).

[62] For Professor Charles Dinarello, see note 48.

[63] Hoffmann-La Roche & Co (founded in 1896) is currently known as 'Roche'. For more details on the company's history, see: http://www.roche.com/about/history.htm (accessed 22 March 2016).

Saklatvala: Then Phil Auron and Charles Dinarello and colleagues cloned the human one, which was for IL-1β (*IL1B*), and Immunex were trying to clone both human forms.[64] Steve Gillis at Immunex had the Dinarello paper to review and rejected it for *Nature*. However, they took the sequence, which they then used to file a patent on IL-1 when a financial milestone with a big pharmaceutical company – who was supporting them – was due. So it appeared that to save their company and their project, they took the *IL1B* sequence from Charles Dinarello and Phil Auron. The affair became the subject of a long legal battle.[65]

Feldmann: I think this is a red herring.

Saklatvala: Red herring, but it's an interesting story.

Balkwill: Walter Fiers also cloned TNF, both mouse and human, didn't he?[66]

Feldmann: That was also from the biotech base. But really, the biotech industry provided the tools, the complementary DNA (cDNA) probes, the antibodies that could be made from the recombinant proteins, and recombinant proteins for looking at human disease tissue. The approach that Tiny and I, and our colleagues, took was to focus on what cytokines were produced locally, so we studied messenger RNA (mRNA) production using cDNA probes. Gordon Duff was interested also in local production; he used immunohistology.[67] Other people looked at cell supernatants and synovial fluid. Every technique was used, and the results were concordant that in the most accessible inflammatory human disease site – rheumatoid synovial tissue – every cytokine possible was detected. This was the basic dilemma, that the initial thinking that knowing what cytokines were present would give you an understanding of pathology and pathogenesis, and, importantly, a potential therapeutic target. This did not happen, and it was actually a very interesting but challenging time to unravel which of many pro-inflammatory and other cytokines might actually be therapeutic targets.

Maini: I think when Marc and I got together and were interested in defining the cells that were producing these cytokines, and what was regulating them, it became evident that macrophages were the producers of TNF and IL-1, but

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[64] Philip E. Auron is a Professor and Chairman of the Department of Biological Sciences at the Duquesne University; http://www.duq.edu/academics/faculty/philip-auron (accessed 22 March 2016).

[65] See, for example, Wolff *et al.* (1986).

[66] Marmenout *et al.* (1985).

[67] For Professor Sir Gordon Duff, see note 57.

interleukin-6 (IL-6) was produced by fibroblasts. We couldn't show T cells producing anything by immunocytochemistry. They seemed to be there as silent cells, but in fact with Marc and Taniguchi, we did some experiments which did demonstrate some IFN-γ in the joint being expressed.[68] We know today that basically these were tired T cells that had been over-stimulated and had gone into a kind of quiescent phase, which didn't mean that they hadn't played an important part, as they'd already done their bit. Basically, we now know that another kind of T cell, the 'T helper 17' (T_h17) cell,[69] and such other cells, are perhaps being the real rascals, whereas people then were only looking at IFN-producing T cells.

I think that the situation was much more complex than we could see in a simplified way, because of the limits imposed by reagents available for study. It became evident that RA was not only an adaptive immune disease, but also an innate immune disease, and I think that was a very important conceptual advance. Then the question was, 'How were they interrelated?' Subsequent work has shown the interrelationships that exist and are orchestrated in chronic disease. It seems that at the beginning of the disease, a more predominantly active part is played by the T cells, and later on the innate immune response comes into being, and, of course, the antibody response is increasingly recognized as important as well. And, although all these immune responses have been demonstrated, the only test that can prove the pathogenic role for any particular molecule is by treatment interventions which block or enhance their activity. That's the only way we could know that TNF blockade works for the treatment of RA, and that IL-6 blockade works well too, and T_h17 blockade works, but not so well. Since we're talking about RA now, we should mention that B cell depletion and blockade of co-stimulation of T cells are also effective. You can begin to see some kind of a sequence with HLA presentation of antigen by T cells setting off a complex response. Maybe the reason anti-T cell activity blocking works actually is because of the blockade, not only of T cell activity, but also of cytokine production downstream. The same thing could be true for the B cell, that the reason B cell depletion or blockade works is because immune complexes are important in perpetuating RA.

Balkwill: And also, B cells make loads of cytokines as well.

Maini: In their own right, yes.

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[68] Buchan *et al.* (1988).

[69] See, for example, Furst and Emery (2014).

Kalden: As Tiny was saying, I think it was exciting work; it was an exciting time. We all had defined new targets for immune intervention in RA patients; the only thing which was really left to us was to prove which one would be the right target by performing clinical trials. Just take the IFN-γ story, for example. In Germany at this time, IFN-γ was used for treating RA patients based on a small bit of evidence. In some of the patients being treated positive changes with regard to signs and symptoms were observed, but after quite a time this trial was stopped since no real differences were seen between patients treated with verum as compared to the placebo.[70] As in the early days, there is still today a search for new targets for the intervention of the clinical outcome of RA. This is because not all patients will respond well to the biologics developed towards the end of the last century.

Another cytokine, which has turned out to be a good target for immune intervention, is IL-6. Of interest is that a French group published, in the early 1990s, data indicating that blocking IL-6 was treating RA effectively. Thus, discussing different targets indicates that there might be different pathways which dictate the clinical picture of RA.

Balkwill: Would you say then that you hit the jackpot, as it were, with TNF, but that it could have been another cytokine target – it could have been IL-6, or was the data stronger for TNF?

Feldmann: I think there's endless potential for speculating how history might have been different to what it was. I believe that I can represent the history as it actually took place, because a lot of what was successful took place in the laboratory that Tiny and I ran at the Charing Cross Sunley Research Centre, as it became part of the Kennedy Institute.[71] I think if we wanted to do revisionist history, maybe that's for a different afternoon.

The key dilemma at the time was which of the numerous pro-inflammatory mediators that could be shown to be produced in the joint: IL-1, as Jerry pointed out; IL-6; TNFα; granulocyte-macrophage colony-stimulating factor (GM-CSF); IFN-γ: these were all known to be expressed in joints by about 1988 – which, if any, of these was a therapeutic target? It was quite interesting that the emerging concept of cytokine redundancy, for some researchers, acted in a

[70] In a trial, the 'verum' is a drug containing an effective substance, as opposed to a 'placebo'.

[71] The Charing Cross Sunley Research Centre was acquired by the Kennedy Institute of Rheumatology in 1992; http://www.kennedytrust.org/history.html (accessed 21 March 2016).

rather negative way, because the production of recombinant cytokines allows unphysiological experiments to be done. You can put in this purified protein on cells or cell lines at unphysiological doses, and you can get a huge amount of biology that may never have happened *in vivo*, because you never get so much of the protein into tissues. So it became clear that for purified recombinant cytokines, the inflammatory mediators appeared to have very similar functions and that was the concept of 'redundancy' that TNF, IL-1, GM-CSF, overlapped in properties quite extensively when studied *in vitro*. Study *in vivo* was not very easy because if you put in a trigger, if you put in your purified signal in a mouse or a human, it doesn't stay a purified signal, lots of activation happens. So the dilemma, really, was to find out what might be the best therapeutic target. Actually, we started in this field with the prejudice, common at this time, that IL-1 might be the right target based on Jerry's point that if you put IL-1 into cell cultures or the knee joint of a rabbit, you get more damage than if you put in other things.

The key experiment was performed by our ex-colleague, Fionula Brennan, who regrettably isn't with us anymore.[72] She was a postdoc at the time, a very good postdoc in fact, and she first worked with Tiny, then came to work with me and she inherited from Glenn Buchan, a New Zealand postdoc who had driven our work on the molecular biological approach to identify mRNAs in the joint.[73] The dilemma was to try and sort out which of these, if any, was the best target. The technology at the time that led to the 'breakthrough', which I think is an appropriate term for what happened subsequently, was really to use cultures of rheumatoid synovium. This is an experiment that's hugely challenging to do now because volunteers who give up their synovium have diminished greatly as patients are treated better. But, at the time, the synovium was available from a number of surgical procedures, and Tiny had organized a very good and effective network to collect this material. It arrived in the lab and the key step was to develop the culture system that mimicked the biology in the joint, and keep the cells alive and functioning for about 6–7 days so you could study the interactions, and the mediators produced. It turns out that my initial PhD project in Australia on optimizing immune cell cultures from the mouse spleen was very helpful.

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[72] For Professor Fionula Brennan (1957–2012), see biography on page 83.

[73] Glenn Stuart Buchan (1956–2008) served as an Associate Professor in the Department of Microbiology and Immunology, University of Otago; see http://www.otago.ac.nz/propertyservices/commemorative-register/otago074977.html (accessed 22 March 2016).

When I started the PhD project in 1969, mouse spleen cell cultures worked very occasionally. But it turned out that, with attention to many details, these cultures could be made to work. So I was revisiting things I had done before, and with Fionula Brennan doing the lab work and a little bit of suggestion from me, she developed a system whereby the cells in the joint stayed alive for essentially about 5–6 days, and kept producing the mediators that were believed to be produced *in vivo.* And so she was able to do the key experiment, which was to measure IL-1 from synovial cultures under different conditions. This was done by a very high-tech test known as 'thymocyte proliferation assay'. There were no biochemical tests, or binding assays sensitive enough at the time to measure IL-1. As immunologists, we used the simplest experiment that Tiny already alluded to, blocking antibodies. By collecting antibodies from the biotechs that had cloned these cytokines, and made antibodies, we put antibodies into synovial cultures, and the breakthrough was made with a rabbit antibody to TNF that we got from Genentech.

I think it's worth putting on the record that the scientific social behaviour of biotech in the 1980s was quite different to what it is today. We obtained, from scientists I knew, a whole range of recombinant cytokines, very precious proteins, with no Material Transfer Agreements (MTAs), no paperwork, no nothing. They were just given to you for the furtherance of science and it's possibly a sign of insipient senility, but one wishes that science worked like this today. Needless to say it certainly doesn't.

Mike Shepard, who was on the list to attend today, was the scientist at Genentech who was in charge of extramural collaborations, and he was very instrumental in giving us a whole lot of reagents, which ended up enabling us to show that the blockade of TNF in cultures was effective.[74] Fionula Brennan showed that in seven consecutive rheumatoid cultures, anti-TNF switched off the production of IL-1 assayed by the thymocyte assay, within two days. Seven out of seven osteoarthritics didn't change, and so that was really the pivotal experiment that showed that blocking one inflammatory mediator, TNF, also blocked another one that was as potent, IL-1, and that gave us a clue that there may be a pro-inflammatory cytokine cascade of TNF driving the production of

[74] Dr Michael Shepard worked in Genentech on the development of monoclonal antibody therapeutics from 1980 until 1992; http://warrenalpert.org/prize-recipients/michael-shepard (accessed 22 March 2016).

Figure 8: Professor Yuti Chernajovsky

other cytokines.[75] That was the first clue, if you like, that TNF was the potential therapeutic target, a single mediator, that, if blocked, might have capacity to change clinical behaviour in patients.

Balkwill: Should we now talk about the commercial side of the story and the clinical exploitation, or should we go back to the cell killing and the cancer story of TNF now, rather than go further with the rheumatoid story?

Chernajovsky: Historically, many of the people who worked on IFN started working on TNF. IFN was a cytokine that was discovered in the 1950s, and there was a very potent biological assay for it that was 'viral replication inhibition'.[76] For most of the cytokines that were cloned at that time, there were not very good assays. The way that people cloned and managed to get the IFN genes was by an incredibly difficult methodology; that was to inject phages into the nuclei of frog oocytes, and assess the supernatant of every oocyte by testing in an antiviral assay. So all the cytokines that came afterwards when they were cloned, they were cloned because certain biotech companies had certain assays that other people didn't have. It was very difficult at the

[75] Brennan *et al.* (1989). For a simplified overview of the main biological actions of TNF in RA, see Appendix 2.

[76] For more details, see Pestka (2007).

time to be able to demonstrate that what you had was something new that had biological action. I also think that some of the reasons why people from the IFN field moved into TNF was because they had a lot of experience in trying to understand the mechanism of action of biological compounds that not everybody had at the time.

Maini: I just wanted to explain that the possibility that biological therapy would be of any benefit in RA was really received with great scepticism, as it would be for any chronic disease. When monoclonal antibodies were discovered in 1975,[77] because they were murine monoclonal antibodies, they were highly immunogenic and because of their immunogenicity the initial thinking was that they would only work – if they worked at all – for acute indications. Of course, mouse-derived anti-T cell antibodies were used in transplant rejection, successfully, for one of the first applications for an antibody therapy.[78] Then we had the technology for developing chimeric antibodies, which were shown to be perhaps less immunogenic. But to produce these in quantities that were required for treating diseases that are chronic in nature was a formidable challenge. When we first thought of anti-TNF antibody use in RA, the major limitation was not only persuading someone who already had such a reagent to allow us to use it, but that they would be able to produce enough quantities for a valid clinical trial to be done. We underestimate, now that the engineering of antibodies has become such a normal phenomenon and kilograms of the material are produced reproducibly, that this was not the case in the 1980s when Marc and I were struggling to move from these pre-clinical experiments into the clinic.

I should also mention that we had this really rather naive idea arising from Marc's laboratory. He had a chap called Pat Gray working with him who had cloned a variant of a TNF receptor, which was highly effective in blocking TNF *in vitro*.[79] We did, at one time, think that the Kennedy Institute would actually be able to produce enough for us to treat patients. Marc tried his contacts with industry to try and persuade them to make this therapeutic for us. And before we go to the next phase, which I'm sure we should hear about shortly, Jim

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[77] Köhler and Milstein (1975).

[78] See, for example, Prentice *et al.* (1982).

[79] Gray *et al.* (1990). Dr Patrick W. Gray is Chief Scientific Officer at BioMmune Technologies; see http://www.biommune.net/board/ (accessed 22 March 2016).

Woody appears on the scene from Centocor who already had such an antibody.[80] I think we should divert for a moment and recall that monoclonal anti-TNF antibodies for clinical use were actually developed for treating sepsis, and we haven't mentioned sepsis until this moment. The work of Bruce Beutler and Anthony Cerami showed in a mouse model experiment that sepsis caused a cytokine storm, and this cytokine storm caused multiorgan failure, which you could prevent by giving anti-TNF antibodies at the onset of the induction of sepsis.[81] So when we were doing the experiments with RA, there were only two scenarios in the clinic: one of TNF as a treatment for cancer and of anti-TNF as a treatment for sepsis.

Feldmann: I think it's also worth remembering that the concerns about antibody therapy were really dominated by cost. So the belief was that the cost of these therapies would not be suitable for chronic use. In fact that probably still is the case, but it hasn't stopped progress. The sepsis hypothesis that Tiny alluded to, had led to a number of companies making anti-TNF monoclonals, and they made this for the mirage of treating 300,000 Americans who were dying annually of sepsis, and that this could lead to a one-shot treatment to rescue them and make a lot of money. It resolved the really huge dilemma of new medicine: that when you discover a therapeutic target, can you actually get a therapy? This particular dilemma had been resolved.

There were about half a dozen different antibodies. We could spend a lot of time talking about the 'Why was it difficult to get British companies to work with us?', but it's inappropriate because it would also take hours. This is where Jim Woody came on the scene to really help us, so Jim should say more about it. Basically, he was the first person we could convince to take our hypothesis seriously, and he and the company he'd joined recently, Centocor, to work with us to move these very interesting laboratory findings into a Proof-of-Principle (PoP) situation and early clinical situation.[82]

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[80] Centocor was founded in 1979, and in 1999 became a wholly-owned subsidiary of Johnson & Johnson. In 2008, Centocor and Ortho Biotech Products merged to form 'Centocor Ortho Biotech', while in 2011 the company acquired its current name: 'Janssen Biotech'. For more details on the company's history and activities, see https://www.janssenbiotech.com/company/history (accessed 16 March 2016).

[81] Beutler, Milsark, and Cerami (1985).

[82] The PoP refers to an evaluation of the effect of a new drug/treatment on specific disease biomarkers, but not necessarily on the clinical endpoints of the disease. Professor Sir Marc Feldmann commented: 'while PoP doesn't need clinical endpoints, they are always looked for, and in our case were very obvious.' Note on draft transcript, 24 May 2016.

Figure 9: Dr James (Jim) Woody

Jim Woody did a PhD at UCL under my tender loving care. He was meant to get Avrion Mitchison to supervise him, but Avrion didn't want to and instead Jim got me.[83] He completed his PhD in the minimum possible time, which is what the medics can do and the science students can't do. The medics know that to survive they have to be organized and if they're going to move to another job they've got to finish precisely on time. He was one of those people, so a really good student to have. He'd come as an American to do a PhD much quicker than possible in the US. Jim, do you want to come in and explain?

Dr James (Jim) Woody (via phone): I believe the pivotal experiment from our point of view was done by Fionula Brennan in Marc's lab.[84] At the Kennedy Institute, Marc and Tiny had put together a very good programme, and they were able to culture human joint tissue and study the cytokine production for several days. That was a very informative set of experiments from our perspective, because any time you have human tissue as opposed to animal models, the human tissues are much more informative. Fionula was able to show that the blockade of TNF eliminated the downstream cascade of cytokines in these cultures, and that indicated to us that it would be worthwhile doing

[83] For Professor (Nicholas) Avrion Mitchison, see note 30.

[84] See note 72.

a clinical trial with an anti-TNF, which we had. Interestingly, we had already done a trial in 30 or 40 sepsis patients where we were disappointed, because the anti-TNF, unlike the predictions, didn't do very much; certainly not the benefit we had hoped. So when Marc and I discussed the experiments Fionula had done plus all the background information you've heard about this morning, it became clear that a trial in patients with RA was warranted. So we arranged to go ahead and do that in about 1992.[85]

Professor Henning Walczak: From my understanding, and I joined the field quite a bit later; I realize you started in 1966, the year I was born. After finishing my undergraduate studies in 1990 I was led into it by another article in *Scientific American* that made me visit some labs at the German Cancer Research Centre where scientists work on these cytokines. Shortly thereafter I started working on cell death for my Master's and PhD theses. Following my time in Heidelberg, I joined Immunex in Seattle and, of course, Immunex was mentioned before in a bit of its more shady aspect. Later on Immunex, of course, cloned one of the TNF receptors, namely 'TNF receptor type 2' (TNFR2) and had constructed an Fc protein that consisted of the extracellular portion of TNFR2 joined to the Fc portion of IgG1. This recombinant protein was able to block TNF. And, of course, Jim Woody – who is on the phone – and whom I also met when, together with Peter Krammer, my PhD supervisor at the time, I went to visit Centocor and talked about the possibility of working together with them on another TNF super family member and its blockade, namely 'APO-1 ligand', or 'Fas ligand' as it's known more globally.[86]

Coming back to TNF, for me the question is: how did the race unfold? At some point it had become clear that TNF blockade could potentially work as a therapy in RA and as far as I understood – at least from my time at Immunex, and I was there in 1996, and 1997, the time when Enbrel was being licensed – Enbrel was the first TNF blocker to be licensed as a treatment for RA.[87] There was, of course, the antibody that Jim Woody and Centocor had been working

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[85] Dr Jim Woody commented: 'Ironically the company was unenthusiastic, but the science prevailed, and we were able to initiate the first clinical trial.' Note on draft transcript, 13 May 2016.

[86] The APO-1 (meaning 'apoptosis antigen 1') receptor is also known as 'cluster of differentiation 95' (CD95), and it is a cell surface receptor that can lead to programmed cell death (apoptosis) upon its activation by the APO-1 ligand.

[87] Etanercept (Enbrel®) was developed by scientists at the Immunex Corporation; Mohler *et al.* (1993). Immunex was acquired by Amgen in 2002. For an overview of the drug's indications, see Appendix 3.

Figure 10: Professor Henning Walczak

on.[88] There was indeed another antibody that was developed just across the Rhine from Heidelberg at Knoll, which was part of BASF, and which was later acquired by Abbott.[89]

Kalden: That was the first humanized anti-TNF antibody.

Walczak: Yes, but my question is how did the race unfold once these different molecules had become available to go into the clinical trials?

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[88] Infliximab (Remicade®) was developed by Centocor (now 'Janssen Biotech'). For an overview of the drug's indications, see Appendix 3. Professor Sir Marc Feldmann commented: 'it might be good to point out two other important contributors; Jan Vilcek at NYU whose lab made the original hybridoma that was genetically engineered by John Ghrayed at Centocor.' Note on draft transcript, 24 May 2016.

[89] Adalimumab (Humira®; trade name stands for 'human monoclonal antibody in rheumatoid arthritis') was collaboratively developed by BASF Bioresearch Corporation (Worcester, Massachusetts) and CAT as 'D2E7', then further manufactured at BASF Bioresearch Corporation and developed by BASF Knoll (BASF Pharma) and, ultimately, manufactured and marketed by Abbott Laboratories after the acquisition of BASF Pharma by Abbott. In 2013, Abbott split into two companies, one retaining the Abbott name and the other named 'AbbVie' (the current owner of Humira®). For more information on the D2E7 development, see Kempeni (1999). For more details on the drug profile, see respective Drug Profile (authored by B. Yang) at the Discovery Medicine website: http://www.discoverymedicine.com/Benjamin-Yang/2009/05/21/drug-profile-humira/ (accessed 15 March 2016). For an overview of the drug's indications, see Appendix 3.

Feldmann: I think it's important to establish the exact chronology of the PoP early clinical trials. This was done in 1992 with what became 'infliximab', which was known at the time as 'cA2'.[90] So this was an experiment that was done with Centocor, with Jim Woody, but not with the professional clinicians at Centocor because they were chasing CD4 T cells as the cure of RA, and they were not as impressed as Jim was about anti-TNF. So from May 1992 through to about July 1992, Tiny treated ten patients, or probably only nine by then.

Since 1984 we have held scientific meetings to help translational research, and we had one of these in September 1992 – it was one of the few that was not at Trinity College (Oxford); this one was in Israel, on the Dead Sea in Arad.[91] We asked Centocor for permission for Tiny to present the results, and in their wisdom they agreed.[92] My friends from Immunex, Genentech, Roche etc., were all present. Tiny disclosed that nine patients had responded really well to anti-TNF therapy. That was an important event for the patients, because it went from being one company half-heartedly pursuing anti-TNF therapy to three or four companies pursuing the same goal with rather larger resources. I think that's been terrifically good for patients. Do you want to amplify, Tiny?

Maini: Yes. I think the scepticism I referred to earlier was quite a dominant, negative force when we wanted to go into man, into clinical trials. A lot of our academic colleagues didn't believe this would work, and might be harmful. There was the question of immunogenicity, cost, and all these factors that were real obstructions, roadblocks to progress. It was the foresight of someone like Jim, who could see maybe an alternative use for a product that Centocor had developed for treating sepsis, that didn't seem to be working in sepsis, that allowed us to do the key experiment. The results were so dramatic that, Jim, you may recall, some of your senior directors visited London and actually cross-examined me in great detail about how good this really was. We had to show them our lab books and our clinical data to prove the dramatic benefit that had ensued, including repeat biopsies in patients that we had performed.

I think that the persuasion was sufficient then, not only to do a very complete, open label trial – open label because we were not at all sure how harmful this could be to our patients, so they had to be supervised with great care and caution – to

[90] See note 88.

[91] For more details about this meeting, see Feldmann (2009).

[92] For Centocor, see note 80.

be quickly followed by a placebo control trial that conclusively showed a dose response to infliximab, called 'cA2' in those days, in RA patients.[93] That was the first multicentre trial in which Joachim Kalden from Germany, Josef Smolen in Vienna, and Ferdinand Breedveld were co-opted by me, because they were my friends, to take part in this trial.[94] They were all experienced in treating RA, so this seemed a very good group to get together. Basically what happened was that a British idea, with European help, was facilitated by an American company. And the PoP was dramatic in this trial as we published in *The Lancet.*[95] We also published a follow-up study that demonstrated that the clinical response only lasted for the duration of the antibody in the blood, and that as soon as the antibody levels fell somewhere below 1 μg/ml, the disease came back.[96] So we then did the obvious experiment, which was to repeat treatment with this antibody and showed the same level of response but in a significant number of patients. In a small number of the patients whom we treated, the duration of response diminished and we could show that this was very likely due to the development of antibodies, blocking antibodies, to the antibody that we were using for treatment.

So that was the score in 1993/4 when, in the meantime, both Roche and Immunex became involved in the race to develop their therapeutic soluble TNF receptors for treating RA. The Roche type 1 soluble receptor fusion protein was very effective, but turned out to be immunogenic as well, and in this case the immunogenicity led to rapid clearance and shortened efficacy.[97]

Actually, one other factor came to light during these trans-Atlantic trials: in Europe, they were showing efficacy but the same product in the USA by Genentech, I think, was apparently not effective. This was a quality-control issue, so Roche was not only troubled by the fact that not only did their receptor fusion protein appear to be immunogenic, but, actually, they were not able to show convincingly its reproducible production.

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[93] Elliott *et al.* (1993, 1994a).

[94] For Professor Josef Smolen, see biography on page 88. Professor Ferdinand Breedveld is Head of the Department of Internal Medicine and Rheumatology at Leiden University Medical Centre; see http://www.articulumfellowship.com/centres/NL_breedveld.html (accessed 22 March 2016).

[95] Elliott *et al.* (1994a).

[96] Elliott *et al.* (1994b) and Maini *et al.* (1998).

[97] Rau *et al.* (2003).

Kalden: Stability was missing.

Maini: Stability of the receptor fusion protein was the other factor. Nobody thought at that point that the Immunex fusion protein would work in RA, because it had low-affinity binding and actually was a dissociable complex. People thought that this would be readily displaced when given *in vivo* and that it would not work very well, and actually it didn't work as well as the antibody but it was much less immunogenic. The claim was made that it was not at all immunogenic. We now know, of course, it is immunogenic but the antibodies are not a significant factor in blockading the therapeutic action. There was one British company, a biotech company, Celltech, that also produced an anti-TNF chimeric antibody that was of an IgG4 class that was in clinical trials soon after the infliximab, cA2 trials that we did, that confirmed efficacy.[98] Now this was the dramatic thing about the anti-TNF trials. Every biologic that was used was effective and the results were strikingly similar: 60 to 70 per cent of patients were responding and about 30 to 40 per cent weren't. As a result, we began to realize that not every rheumatoid patient responds, but the reproducibility of the phenomenon was a huge boost to the development of these antibodies. First in the clinic for RA was the Amgen product in 1998 because, and Jim may like to comment on this, Centocor just did not have enough money to do the big trials we needed to do in RA.[99] Instead, they focused on Crohn's disease and they got a licence for Crohn's before anybody else, because, in fact, the Amgen anti-TNF did not work in that disease.

Kalden: Could I just add, around the same time, in 1993/4, Joachim Kempeni, working at a little company in Germany called 'Knoll' that was owned by BASF, produced a human anti-TNF monoclonal antibody.[100]

Maini: No, no, no, it was produced by Cambridge Antibody Technology (CAT), by Greg Winter.[101]

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[98] Rankin *et al.* (1995).

[99] Etanercept (Enbrel®) was approved for use in RA in 1998; see Appendix 3.

[100] See note 89.

[101] Cambridge Antibody Technology (informally known as 'CAT'; founded 1989) was a biotechnology company that focused on antibody therapeutics and that was acquired by AstraZeneca in 2006. Sir Gregory Winter was one of the founders of CAT and is currently the Master of Trinity College, Cambridge; http://www.trin.cam.ac.uk/node/353 (accessed 22 March 2016).

Kalden: Whoever produced this antibody, the small company Knoll was sold by BASF to Abbott.

Feldmann: The protein was made by CAT. That's what the lawyers think and that's what hundreds of millions of royalties paid by Abbott to the Medical Research Council (MRC) says. BASF commissioned the antibody from CAT, but BASF was bought by Abbott.

Kalden: No, no, BASF is still alive. It's a big company. Knoll was basically part of BASF and then they sold Knoll to Abbott.

Feldmann: That is right.[102]

Maini: Knoll developed the CAT antibody clinical trials.[103]

Walczak: If I'm not mistaken, on the basis of the fact that scientists at Knoll had purified human TNF receptors, and I'm not sure whether it's true but I heard it was actually purified from urine. Does anyone know whether that's true or not?

Balkwill: Wasn't it that the Italians used to collect urine from nuns to purify hormones, and Alberto Mantovani would tell you that they used this urine to purify the TNF receptors.[104]

Feldmann: The TNF receptor purification that led to patents was done in Israel.

Balkwill: By David Wallach.[105]

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[102] Professor Henning Walczak commented: 'in the meantime I have found out that indeed CAT acted as CRO (Contract Research Organisation) for BASF/Knoll in the generation of a humanised antibody against TNF and, as a function thereof, receives royalties on sales which, even though they were kept at a rather low percentage rate at the time, amount to massive sums because of the high sales of Humira […] it [Humira] was developed by BASF/Knoll with the help of CAT who acted as a CRO […] so far I have not been able to receive any official statement from BASF/Knoll or anyone involved on their side at the time, but I think the clarification I provide above should suffice to make the point within our document.' E-mail to Dr Apostolos Zarros, 8 June 2016.

[103] For more details, see Lorenz (2002).

[104] Professor Alberto Mantovani is Scientific Director of the Humanitas Clinical and Research Center and Professor of Pathology at the Humanitas University, Italy; http://www.humanitas-research.org/mantovani-alberto/ (accessed 22 March 2016).

[105] David Wallach is Professor at the Department of Biomolecular Sciences at the Weizmann Institute of Science; see http://www.weizmann.ac.il/Biomolecular_Sciences/Wallach/ (accessed 22 March 2016).

Feldmann: David Wallach and the Weizmann Institute of Science group; we have to assume that the royalties they get on the patents mean that that is the correct history.[106]

Walczak: Yes, but I know that there are employees at Knoll, who are now at BASF, of course, who get royalties for having contributed as inventors to the development of the anti-TNF antibody now known as 'adalimumab' or 'Humira', so there must be some connection with respect to what they have done on this as well.

Woody: Just to comment on some of the history: interestingly, Centocor was the first one to ever inject an anti-TNF antibody into humans. As Tiny pointed out, there was a lot of scepticism that this might be extremely toxic, and so we did a lot of primate studies to be sure that it wasn't harmful, and it wasn't.[107] And in the sepsis patients that we treated, while they didn't get better, they didn't get worse either. So we had fundamental data for the first time that would allow us to go into rheumatoid patients. At the time there might be another product from Roche/Genentech, lenercept,[108] that was a very potent anti-TNF inhibitor, but the fusion protein had a construct in it that turned out to be extremely immunogenic. So they had a hard time with that molecule for a long time.

As Tiny pointed out, we were also conducting an anti-CD4 trial in patients with RA and also multiple sclerosis (MS), which eliminated T cells, but it failed, even though at the time it was thought that T cells were driving the disease.[109] There are lots of reasons why people thought we eliminated regulatory T cells; not quite sure what the answer was to why the anti-CD4 trial failed. But all of this was going on; it was a very productive time for sure. We enjoyed the connection with Marc and Tiny, and also Joachim Kalden, Professor Josef Smolen, and

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[106] Engelmann *et al.* (1989). Note that in this original article of the Weizmann Institute of Science (where David Wallach is the senior author), the assistance of the Cesare Serono Research Institute (Rome, Italy) for 'preparing the crude urinary proteins' is acknowledged.

[107] Dr Jim Woody commented: 'the primate studies would have been confidential Centocor information submitted to the FDA with the cA2 Investigational New Drug (IND) submission. As I recall there was no significant toxicity identified with cA2 administration to the primates, which allowed Centocor to move forward with the initial clinical trials in patients with sepsis.' E-mail to Dr Apostolos Zarros, 12 May 2016.

[108] Lenercept is a fusion protein of the type I TNF receptor (p55) and a human Fc IgG.

[109] The Lenercept Multiple Sclerosis Study Group and The University of British Columbia MS/MRI Analysis Group (1999) and van Oosten *et al.* (1997).

Professor Ferry Breedveld.[110] Interestingly, they put a few patients with psoriatic arthritis on this trial and their psoriasis went away, so we knew that the antibody would work in psoriasis. And Sander van Deventer in Amsterdam treated a Crohn's patient with cA2, and that person got well, so Centocor actually got the drug approved for treating patients with Crohn's who had fistular disease, which was the most rapid track for approval. It was only later, after Enbrel was approved, that Centocor got approval for RA and psoriasis, so there was a bit of history there that is interesting.[111]

Feldmann: One of the things that's really important is to try and connect the history of the concepts as they move from the lab to the clinic. So one clinical trial that's underappreciated was the follow up of the open-label study. The open-label study grew to 20 patients, seven of whom got retreated a number of times. The importance of a retreatment study, which Tiny has already mentioned, was that some of the patients were retreated multiple times, four or more times, and they had multiple responses. What this demonstrated conceptually was that on repeated blockade of TNF, as the patients got better again, they were still running exactly the same mechanism. This TNF-dependent effect on other cytokines that could be demonstrated in humans from the clinical trials – for example, anti-TNF treatment of patients reduces serum IL-6 within a few hours[112] – this was a formal proof that this TNF-dependent cytokine cascade, first found in Fionula Brennan's cultures, extends *in vivo* to patients. The retreatment success over a year, but reduced duration due to immunogenicity, validates the idea that this TNF-dependent cytokine cascade was operative and relatively stable with time. This is unlike cancer, where pathways are much more likely to change quite quickly. Of course, this is an underpinning of the use of antibody therapies chronically, because if the pathways are relatively stable you can predict that the sales or the use of monoclonal antibodies in cancer is for a much much shorter period than in chronic inflammatory diseases like RA.

Maini: I think one of the important underpinnings of the clinical trial's finding was the mechanistic studies we undertook at the same time.[113] That was unusual in a clinical trial. I don't think there had been any clinical trial that I'm aware of

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[110] For Professors Josef Smolen and Ferdinand Breedveld, see note 94.

[111] See Appendix 3.

[112] Charles *et al.* (1999).

[113] Maini *et al.* (1995).

in which serial biopsies were done on patients' joints, and serial samples from the blood were studied, as we did, to demonstrate a dramatic reduction in IL-6 within hours, as Marc has alluded to, but also, within days, a change in the morphology of the joint's pathology. When we repeated the biopsy of joints, we found difficulty because, instead of being a large, boggy joint with a lot of tissue, the joint had shrunk, there was very little fluid left in it. When you took the biopsy there was a dramatic difference; it was no longer so cellular. So it was quite obvious to us, very early on, by 1993/94, that a major effect of anti-TNF was on cell trafficking.

Balkwill: I can remember, because I was very inspired by your work when thinking about anti-cytokine therapy in cancer, especially by the fact that you were doing these clinical experiments on patient samples during a trial. But the one paper that I remember was where you actually took leukocytes from the patients and you radiolabelled them and then studied their trafficking, or not, into the joint in the presence of anti-TNF.[114] I will never forget that experiment. I've always been interested in translational experiments, doing experiments in patients, and to see you doing something like that was really inspirational.

Maini: Just to clarify that: in fact, when we found this lack of cellularity, the hypothesis was that either the cells were dying or there was an effect on the trafficking of the cells into the joint. And to investigate it, what was done in collaboration with people at the Hammersmith Hospital, who were good at imaging joints with labelled cells, was to label polymorphs with indium-111, reinject these into RA patients, and you could do a γ-camera imaging of the knee joints and the hands, and we could show a dramatic difference in the retention of the radiolabel before and after treatment.[115] It was these kinds of experiments that were really underpinning that this was going to be a very effective attack on the pathogenesis of RA.

Balkwill: But weren't they also pioneering in general, because people in any sort of clinical trial, in cats or anything else, I don't think at that stage that people really did try doing repeat biopsy? We struggle enough now.

Maini: I think that is correct.

Balkwill: But to me they were quite pioneering. And you also showed a reduction in matrix metalloproteinases (MMPs) as well, didn't you?

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[114] Taylor *et al.* (2000).

[115] Taylor *et al.* (2000).

Maini: MMPs and other cytokines.

Balkwill: When we, much later on, managed to persuade Centocor to do some studies in cancer patients with anti-TNF, the three things that really I thought of from the rheumatoid studies that you did were that: (i) the anti-TNF reduced other pro-tumour cytokines like IL-6; (ii) it reduced leukocyte trafficking into joints – as you know leukocytes trafficking in tumours is also a problem; and (iii) it also reduced MMPs and chemokines, which were involved.

Williams: Both Marc and Tiny have alluded to the second clinical trial in which patients were retreated with anti-TNF when the disease flared, and it was observed that patients developed antibodies to the chimeric antibody cA2 and it was hypothesized, as Marc said, that this limited the duration of the therapeutic effect. By then I was involved in pre-clinical studies in mice, and I had looked at the effect of TNF blockade in an animal model and it was found to ameliorate collagen-induced arthritis, but, as in patients, the duration of effect was limited, because the disease flared as soon as the antibody was cleared. And also, using hamster antibody, the mice developed antibodies to the injected hamster anti-TNF antibody. To try and kill two birds with one stone, we looked at the effect of combination therapy, using anti-TNF with anti-CD4, and it should be remembered that anti-CD4 was being tested around this time in a number of clinical trials. It was felt that by targeting the T cell response as well as the pro-inflammatory TNF mediated response, you could get a more sustained therapeutic effect. There was the additional benefit of actually preventing the antiglobulin response to the foreign antibodies that were injected. That really formed the basis, at least in part – I'm sure Marc and Tiny will comment on this later – of the rationale for the combination of anti-TNF with methotrexate; methotrexate being a drug that was in common use and that had an effect on T cell proliferation, and therefore was considered perhaps to be an alternative to anti-CD4 but with the same concept of targeting the immune response as well as the pro-inflammatory response mediated by TNF.

Kalden: Tiny, I remember that weekend very well. We were in your home and we discussed the combination therapy. The discussion was centred on the issue: should we really combine the monoclonal antibodies with methotrexate? After a long discussion, we all agreed to combine a rather low dose of methotrexate, but it really took us the whole weekend until we decided to go ahead to see if this combination would be better than a monotherapy with either of the

Figure 11: Professor Sir Ravinder Nath (Tiny) Maini (left) and Professor Henning Walczak (right)

drugs alone.[116] In addition, the charm of this study was that we decided to do some parallel scientific experiments with regard to the mode of action of the monoclonal antibody, looking at the blockade of different pro-inflammatory cytokines in the periphery, at acute phase proteins, and at the cell infiltration of the synovium. Thus, at the end of these experiments we had quite a good knowledge of how and where a blockade of TNFα would be efficacious in RA patients.

Balkwill: I guess we're around 1994 now. Marc, Tiny?

Feldmann: So I think the dilemma in 1994 was how this would enter rheumatological practice. The trials till then had been quite short-term, and short-term trials in a lifelong disease don't have much impact. So Richard already introduced the idea that in mouse models combination therapy could be used, and this could be an improvement on anti-TNF alone. What Richard [Williams] did first was a blockade of CD4 T cells on top of anti-TNF.[117] That became a precursor to the most effective trials, which were combining low-dose methotrexate with anti-TNF. I think Tiny is probably best placed to discuss that.

Maini: It's important for us to put in perspective both the positive and the disheartening side of the story in 1994. At that time, the results of the Immunex product that you heard about actually weren't known because they hadn't done any trials. The only anti-TNF agent that had been used in patients to date

[116] Maini *et al.* (1998).

[117] Williams *et al.* (1994).

was the Centocor anti-TNF in RA with a placebo-controlled trial,[118] and the repeat therapy trial,[119] which showed that immunogenicity was going to be a limitation. So the question was whether we could devise a way of overcoming the immunogenicity for prolonged therapy, and initially we thought the best way would be to block T cells. But because Centocor had developed a CD4 antibody as well as the anti-TNF antibody that we were using, and the anti-CD4 had been in clinical trials in RA patients, it seemed obvious to Marc and me that we should use that combination in RA patients. But Centocor was very reluctant to do a combination trial. I think the reason for this, and maybe Jim can explain, was essentially to do with the fact that you couldn't use two unlicensed products simultaneously, so that path was closed. The only well known anti-T cell drug that was in use at that time was cyclosporine, and that had been used in transplantation very effectively for preventing rejection. Indeed, there had been some early trials in RA showing that it had limited efficacy, so one of the things that Richard Williams did was to test the combination of anti-TNF with cyclosporine and he showed – I think this followed the work with anti-CD4 – that that also was effective.[120]

We looked around for the possibility of using that combination, and engaged in correspondence with Jim and Centocor about a trial using that particular combination.[121] But it soon became apparent to me, as a clinician, that there just weren't enough patients on cyclosporine and that it was too toxic a drug to expose patients to in a blinded trial, when you hadn't any hope of keeping patients on the active principle cyclosporine truly blinded, as they developed hypertension and renal problems, and so, it would be very obvious to the clinical trialists which patient was receiving what. The problem was that the effect of anti-TNF was so profound that the concern was how would we demonstrate an additive effect, and how would we design a trial that actually showed this synergy or additional benefit? Well, having considered the obvious cyclosporine, we then had to scratch our heads and wonder what else we could use. I think in 1993 or 1994, Marc and I were writing to Centocor about long-term strategy and we proposed methotrexate mainly because it was commonly used, and we knew that it had a profound effect on innate immunity. All the work that

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[118] Elliott *et al.* (1994a).

[119] Elliott *et al.* (1994b).

[120] Williams *et al.* (1998).

[121] For Centocor, see note 80.

was published to date on humans with methotrexate was that it was a strong inhibitor of polymorph function and of prostaglandins, and I think the feeling was that maybe here lay something that might be an adjunctive.

There had been, at that time, no published work on methotrexate and adaptive immunity in man, to my knowledge. After we had designed the combination with methotrexate, because that seemed the most practical thing to do, it became clear to us and confirmed by a publication that arose, I think from France, that actually it did inhibit proliferation of T cells in humans.[122] This was quite encouraging, but by then we had already embarked on this combination trial and, as Joachim Kalden reminded you, the exact construction of the trial required a lot of thinking. One of the principal concerns was that we didn't do any more harm to these patients on anti-TNF. By then, we knew it was an immunosuppressive drug and especially caused the unmasking of tuberculosis, and we had also seen one patient with a lymphoma, so there was concern that this combination could prove to be more toxic. And the issue was, if we were going to use methotrexate, what dose should we use? We decided, after a long, long discussion that we had to go with the lowest possible dose. Now, let me remind you that methotrexate works from the accumulation of polyglutamates of methotrexate in the cell, and that blocks a number of pathways. The hope was that patients who were already on methotrexate and were showing continuing disease activity would have enough saturation of polyglutamate in their cells for any synergistic effect that we might be looking for; that's how it turned out to be with the trial.

In 1994 we began a multicentre trial, and it was completed by the end of 1995. We had the first data in our hands in 1996. When Jim Woody, Marc, and I saw this data, we were really astonished. What it showed was that anti-TNF on its own in three doses worked, and, interestingly, at the highest dose of anti-TNF, which was 10 mg/kg given repeatedly, immunogenicity was suppressed. There was hardly any immunogenicity at that dose. We were reminded, when we saw that data, of experiments that had been done in the 1960s by Av Mitchison and others on inducing tolerance to soluble immunoglobulin;[123] that we were basically reproducing in man a similar kind of tolerogenic effect. But we know from their basic studies in mice, that this is short-term B cell tolerance, and it's very quickly reversed. So the option from this data was either the highest

[122] Genestier *et al.* (1998).

[123] Mitchison (1964).

dose of infliximab that would work long-term, or that the combination might work. The lowest dose of anti-TNF that was used was 1 mg/kg, and, at that dose, with 7.5 mg of methotrexate weekly, we saw a profound beneficial effect. Indeed, we also saw a reduction from 50 per cent immunogenicity to about 3 per cent immunogenicity. For the first time it was apparent, I think, that low-dose methotrexate, a minute dose, was actually a primary immune response suppressive agent. That was very encouraging, and that was only one of the reasons that that combination was working, because quite clearly it was also working in patients in whom no immunogenicity had been induced, and the combination was showing a very profound effect.

To this day, I don't think we really understand why a combination of methotrexate and every anti-TNF agent that has been tested, subsequently, shows an enhanced benefit. So, to cut a long story short, Centocor-based antibody trials with methotrexate were the first, and it was the first combination to be licensed, although Immunex got the licence for monotherapy first;[124] they accelerated the work into clinic without having done any of the pre-clinical work, without having done any mechanistic studies. They just did clinical trials at high speed and at high volume in the USA, to prove that they had a very good therapeutic. They were in the clinic a year before Centocor, who meanwhile was trailing behind because they didn't have enough money for RA trials of the size and design we wanted them to do. We said that, really, it's no good just showing an anti-inflammatory effect, you also have to show a disease-modifying effect, and disease modification, as laid down by the Food and Drug Administration (FDA) and the regulatory authorities, required a two-year trial. And the read-out was X-rays of the joints which show damage changes. That was the reason that this trial that we then performed for the Phase 3 development with Centocor took two years, and the licence had to wait, I think, until 1999 because of this delay.

So just to step back: the first combination therapy, the results of which we already saw at the end of 1995, early 1996, was when we realized that this could be a patentable finding. Publication was partly held up for that reason, but partly because, when we submitted this trial for publication, initially to *The New England Journal of Medicine (NEJM)*, and then to *The Lancet*, it was turned down. Eventually, we had to go to *Arthritis & Rheumatism,* which is the most significant rheumatology journal, and we had three revisions before they accepted this trial because they kept on asking questions, being rather

[124] See Appendix 3.

disbelieving about the statistics or the findings.[125] We had to go through several iterations before we could really nail it down that this truly was a very genuine, and very good, finding. When that trial was published, all other companies went for the combination therapy and very soon we had the Immunex (later Amgen) product etanercept with combination, and then adalimumab,[126] which is the Abbott fully human antibody, which, of course, being less immunogenic followed. This drug in combination with methotrexate did not rely on suppression of immunogenicity, although that is a factor, and there are many people who have been studying the effect of immunogenicity of so-called 'fully human antibodies'. They are immunogenic and there is no doubt that patients who develop antibodies have a significant reduction in their response. In a nutshell, I think we can conclude that, from 1994 to 1998, it was possible for us to show that combination of an anti-TNF monoclonal antibody with methotrexate, when used sequentially in patients with uncontrolled disease, was a very sensible way of treating RA, because methotrexate is a very effective drug as monotherapy. When methotrexate is given as monotherapy, 50 per cent of patients will respond very well, especially if it's introduced early in the disease, and such patients don't need any other drug; they are restored to a very good quality of life and can tolerate the drug very well. It's the patient who isn't in that good category that is an ideal patient for treating with combination therapy with anti-TNF. Our trial design actually demonstrated that because we treated these patients on methotrexate without interrupting treatment, in other words with no washout period, the combination ensured that there was enough bioactivity of the polyglutamates in these patients to show the combination actually was due to a methotrexate effect.

Balkwill: So is that the same basis for treatment now? What's changed since then, or is it still very much accepted?

Maini: Nothing has changed. The only thing is that the combination is now being used in the USA from the initial stages of the disease. So if you have very serious disease, rheumatologists will say 'This patient needs combination therapy from the start.' There is a school of thought that combination therapy might be better for RA and there are some trials which purport to show that; however, our friend Josef Smolen, who isn't here today, has written some very

[125] Maini *et al.* (1998).

[126] See note 89.

excellent papers that suggest this is unnecessary.[127] The European League Against Rheumatism (EULAR) has published recommendations on how to treat RA and they have analysed the information in the clinical trial domain.[128] The conclusion is that actually methotrexate alone, if used initially in combination with corticosteroids, is just as effective as methotrexate plus anti-TNF.

Woody: I can add some clarity to Tiny's comments. At the time, to use two experimental drugs was very difficult just because of the regulatory hurdles that you'd have to get over. I think, unlike a lot of others, we were very open in terms of discussing the toxicity associated with anti-TNF and made it available to everyone in the field at meetings and otherwise, and we knew that there was a risk for infectious diseases. There was a concern about lymphomas too because we had had a case.[129] We didn't want the drug to go on clinical hold because it was providing benefits. So things like cyclosporine would have been one of the agents, along with anti-TNF, that would put people, we thought, at higher risk. So the use of small doses of methotrexate seemed a reasonable way to go and that was a major achievement that Tiny and Marc produced in terms of reducing the human anti-chimeric antibodies and allowing repeated doses of the drug, which is actually the same exact formulation that's given today. So congratulations to them on a major, major achievement, including the damage of the joints being reversed.

Feldmann: I just want to add two things to what Tiny's said. I think some of the difficulties in publishing the paper was from the nature of the trial.[130] There were seven arms with about 15 patients per arm, and the statisticians that *NEJM*/*The Lancet* use have quite different paradigms for what is a proper clinical trial. So, for example, in cardiovascular disease they've insisted on thousands per arm so we were some way away from optimal numbers. It's not that we

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[127] See, for example, Smolen, Aletaha, and Keystone (2005). Professor Josef Smolen was unable to attend the Witness Seminar. See also note 94.

[128] Smolen *et al.* (2014).

[129] Dr Jim Woody commented: 'Tiny will recall the first disclosure of this issue. It was complex, as the patient had been treated with many potent cytotoxic drugs for RA over the years, and had a previous lymph node biopsy for possible lymphoproliferative disease, read out as negative. It caused us to survey the RA data for the incidence of lymphoma in RA patients, which was much higher than the general population. So while we were cautious, and disclosed the issue, at the time it was unclear whether there was a correlation or not. As data became available, there was an obvious lymphoma risk to be weighed against the joint destruction benefit.' E-mail to Dr Apostolos Zarros, 12 May 2016.

[130] See note 125.

wanted to do 15 per group, but that was the best compromise because there were three different doses of anti-TNF, with or without methotrexate, so that adds up to seven arms. I think that was a major drive of the publication's bias; the statisticians were probably trying really hard to show that the *P* values were probably statistical artefacts. I'm partially joking, but I think it's challenging to try and publish things in clinical journals when you don't obey the standard criteria or rules for what they would like to receive. But with methotrexate, I think the combination therapy use reveals a very important paradigm, which is, that for biologics to sell well, they've got to fulfil an obvious unmet need. At the time, the obvious unmet need was 'what do you do with patients that no longer respond well enough to methotrexate?' So, in this particular situation they can go straight onto an anti-TNF, and I think that's been beneficial, since, as well as its efficacy, the simplicity of what you're meant to do helped considerably to drive the sales and acceptance, because you weren't asking the challenging thing of patients and physicians to stop existing therapy, you were just adding on to existing therapy. From the historical point of view, it's worth emphasizing that anti-TNFs were the first antibodies to be used for chronic diseases and the first antibodies that sold in very large volumes. The sales are now pushing 30 billion dollars a year. Currently, it is the most profitable drug class; it probably will get overtaken by Gilead's treatment for hepatitis C if they get their own way on pricing, but that's not going as well as they would like.[131] Anti-TNF sales have had a major impact on the pharmaceutical industry. When we started with this project, big Pharma was mostly totally uninterested in antibodies as therapeutics. The attitude to our work with TNF blockade was, 'this is going to be wonderful PoP, we'll find some small molecules that will do the job better, and cheaper.' And that, of course, hasn't happened. So really, what we've had first with anti-TNF, but then with other antibodies, is a change in the way the pharmaceutical industry works.

All big Pharma now sell and work with antibodies. Any of you who have type 1 diabetes will know that repeated insulin injections is what you get and it works fine, and patients don't complain. They're not clamouring for pills because the insulin keeps them well and alive. So now, that paradigm, well understood by diabetics, but not previously understood by the chemically based pharmaceutical industry, has changed considerably and that's a very major step forward because

[131] Gilead Sciences (founded in 1987) is a biopharmaceutical company focused primarily on antiviral drugs; http://www.gilead.com/about/corporate-history-timeline (accessed 22 March 2016).

Figure 12: Associate Professor Richard Williams (left) and Professor Sir Marc Feldmann (right)

it's enhanced the repertoire of potential therapeutics. It's much easier now for small companies to develop biological therapeutics, as the risk of failure is lower than the much more challenging production of small molecule therapeutics.

Walczak: In terms of lessons learnt, it's totally clear to me that at the beginning of the journey for anti-TNF antibodies, like you said, Tiny, it seemed unachievable clinically to get the required amount of antibodies produced. You were able to show that the concept works and subsequently the pharmaceutical and biotech industry, or the biotech industry, like Jim Woody and others, have shown that they can then deliver the antibodies if it's required in the end; it can be done. I think the lesson to be learned from that for us as basic scientists interested in finding new ways to treat diseases is: don't let yourself be talked down by people telling you, 'Oh, we cannot make this into a drug.' For example, right now there are, as you know, a number of therapies – apart from the cancer immunotherapies that are antibodies that work very nicely – which are based on antibody therapies that have worked in the TNF field. Now we can develop antibodies, and the pharmaceutical industry has accepted a number of cellular therapies like chimeric antigen receptor (CAR)-expressing T cells or T cell receptor (TCR) transgenic T cells; these therapies work extremely well in certain cancer entities yet the pharmaceutical industry, at least a major part of it, is still saying: 'That's going to be very difficult to translate, this is probably for academics to do it in certain centres, but we will never be able to translate that into major drug-like therapies'. I think you have shown that this statement is probably wrong, that we can take anything that works – if it works well enough

– into patients and that it can then also be produced effectively in a process that is acceptable to the FDA and European Medicines Agency (EMA), as it has been with antibodies. I'm pretty sure, I'm actually certain, that it will be possible to achieve that also with CAR- and TCR-transgenic T cell therapies. And that, for me, right now is the major lesson that I have already learnt today.

Balkwill: Just coming back to RA, I think, one stimulus for this explosion of new biologics is also that, in spite of this tremendous progress introducing the anti-TNF in the therapeutic repertoire for RA patients, only two-thirds of patients do respond and some of those patients only partially. So there was the need, and there is still the need, to develop more biologics and discover new targets, and more vehicles to target these new targets. In addition, of course, they saw that as something which brings money if it's successful. We have now more than 300 molecules being tested in trials for immune intervention.

Williams: I just wanted to mention one other point, that clearly anti-TNF therapy has been extremely effective, but most cytokines are highly pleiotropic, and back in the 1980s there was a literature, mainly by Hugh McDevitt and Chaim Jacob in the States, showing in fact that administration of TNF was actually beneficial in animal models of, I think it was SLE and type 1 diabetes.[132] There was also at least one clinical trial in MS where anti-TNF therapy actually exacerbated the disease.[133] So I don't think this story is completely finished and, in fact, part of our current work is really addressing this question: what are the different roles played by TNF and in particular on the immune system? In fact, TNF is immunosuppressive and we're currently investigating the molecular mechanisms involved, so I don't think this story's finished.

Balkwill: I think that's a very good point, and something that maybe allows us to just divert off for a minute and think about cell death, cancer, and things like that.

If I could just step back in history a bit, because not only was TNF given to mice as a therapy for autoimmune disease but, as we all know, TNF was given to treat animal cancers.[134] And if you go back to Coley, probably Coley's toxins did not induce just TNF, they might well have induced TNF amongst other

[132] See, for example, Jacob and McDevitt (1988).

[133] The Lenercept Multiple Sclerosis Study Group and The University of British Columbia MS/MRI Analysis Group (1999).

[134] For more details, see Balkwill (2009).

things, but probably some beneficial T helper 1 (T_h1) response, which we would understand a lot more now in our new knowledge of cancer therapy.[135] But if we go back to when the first TNF was purified by Carswell and Old in 1975, and it was a *Cancer Research* paper, wasn't it?

Saklatvala: That was a *PNAS* paper, the one with the spectacular photographs of the assay showing the haemorrhagic necrosis; that was 1975.[136]

Balkwill: No, this was the purified protein, it wasn't the cloned molecule, but for at least 10 years it was thought that TNF was toxic to malignant cells and not normal cells. Certainly, when we got hold of recombinant TNF from Walter Fiers in 1984/85, it was very clear that it wasn't toxic to many cancer cell lines. As you said, in the small print it was clear that the majority of cells that were killed by recombinant TNF had been pre-treated with actinomycin D.[137] They were killed because the cells couldn't make all those protective molecules that we know so much about now. In fact, the idea that TNF was specifically toxic for malignant cells and not normal cells, and this was the reason why it was going to be such a successful treatment, was based on very flimsy evidence.

Walczak: I think that is important, and you are absolutely right, Fran, that TNF, of course, turned out to be a molecule that is capable of driving gene activation more so than cell death. However, there are different signalling complexes that can drive either gene activation and cell death, and the first molecule that was cloned, which plays a role in TNF receptor signalling, was the receptor interacting protein 1 (RIP1), which is a kinase that was neglected at the beginning. So 'RIP kinase 1' was only called 'RIP1' throughout the literature since it was cloned, until a few years ago, when it turned out that RIP1 is a central molecule for the gene activation but also for the cell death arm that, when there is some perturbance in the gene activation, you can then get cell death. And what people hadn't realized until a few years ago was that TNF-driven inflammation was always thought to be only driven by the gene activatory arm.

People were trying to develop, as you mentioned, Marc, small molecule inhibitors and the nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) was one of the drivers of this TNF-induced gene activation, so people thought they'd be able to develop inhibitors of NFκB, or inhibitors of

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[135] See note 49.

[136] Carswell *et al.* (1975).

[137] Creasey *et al.* (1987) and Flick and Gifford (1984).

Jun kinase (JNK), which is activated on another TNF-induced gene activatory arm, to treat inflammatory diseases. But that never really worked.[138]

As it turns out, in many cases, you cannot block TNF-induced inflammation via the gene activatory arm. And that was, of course, a conundrum. It wasn't until we showed a few years ago that if you have deregulated gene activation and at the same time get aberrant cell death in mice that are devoid of one of the components of the TNF receptor signalling complex, then you get an inflammation despite having less gene activation.[139] So how does that work? We then showed that it's in fact the TNF-induced cell death that is causative for TNF-induced inflammation.[140] So rather than TNF-induced inflammation always being driven by the gene activatory arm, it's now also clear that when you have some perturbation in the TNF-induced signalling that leads to aberrant cell death, this cell death can also result in an inflammation. That can be blocked, and now I'm coming back to the central molecule, by inhibiting RIP1 kinase. So right now the thought is, perhaps at least in diseases where you have RIP1 kinase-dependent aberrant cell death, that you could interfere with that arm of TNF-induced inflammatory processes by inhibiting RIP1 kinase. The first clinical trials are now happening with RIP1 kinase inhibitors that are being developed by GlaxoSmithKline (GSK)[141] but also, of course, by a number of other companies. It will be extremely interesting to see how this will unfold.

Maini: For what indication?

Walczak: I'm talking to different people at different companies about this but it's clear that they would first like to go into more acute diseases but they also think that there is a possibility of going into the diseases where possibly TNF inhibitors work now. I think it remains to be seen whether there are two classes of autoimmunity, so to speak: one driven by TNF-induced aberrantly enhanced gene activation, where that class of drug probably wouldn't work, and the other one driven by TNF-induced aberrant cell death. So it could be that there are two aetiologies of TNF-induced inflammation: one driven by enhanced gene activatory capacity, and one by aberrant cell death that happens. And possibly the RIP1 kinase inhibitors would only work in one arm, but that still remains to be seen.

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[138] See, for example, Liedtke *et al.* (2002).

[139] Gerlach *et al.* (2011).

[140] Gerlach *et al.* (2011) and Rickard *et al.* (2014).

[141] GSK is a pharmaceutical company that was established in 2000 through the merging of Glaxo Wellcome and SmithKline Beecham; http://www.gsk.com/ (accessed 22 March 2016).

There is a lot going on in this field right now. I recently organized, together with Peter Vandenabeele[142] – who, of course, studied with Walter Fiers, whom you mentioned, Fran – the TNF conference in Ghent,[143] and it was fascinating to see how alive TNF research is, not only on the cellular level in order to understand the signalling but also what we're trying to achieve with new insights on the clinical side. Also, with other TNF superfamily members, of course, because what we haven't spoken about so far today is that TNF is the founding member of a whole family of cytokines that, either if blocked, or by sometimes activating their receptors could be therapeutically very beneficial.[144] It looks like this family is full of therapeutic opportunities that can still be harnessed a lot better than we've done so far, and today it appears that TNF was only the beginning.

Dr Marcos Vidal: It is time also to mention the conundrum of TNF as a target and a therapy, and perhaps Fran could discuss whether your own work on the papilloma model, which two roles of inflammation seem to be more promising, and these kinds of parallels where TNF is actually required for tumour progression.

Balkwill: Sure, yes, and also the fly work that maybe explains the conundrum a bit.[145]

When we first got TNF from Walter Fiers, it did cause necrosis of tumours on a good day, there was no doubt. This was looking at the crude models we had at the time, which were subcutaneous xenografts, if I remember correctly. I think we published a first paper with Walter Fiers in 1987,[146] and in 1988 I'd become very interested in ovarian cancer and, also, I felt that subcutaneous xenograft models were not the best model for cancer. We used to grow these xenografts intraperitoneally, and Saleem Malik, a clinical fellow in the lab, was doing a study of treating them with TNF, and we didn't have any other way of really imaging the mice. We would take a few mice every week and look at what was happening to them, and what we found was quite extraordinary: that actually the TNF was

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[142] Dr Peter Vandenabeele is a Group Leader at the Flanders Institute for Biotechnology (VIB), Belgium; http://www.vib.be/en/research/scientists/Pages/Peter-Vandenabeele-Lab.aspx (accessed 22 March 2016).

[143] The 15th International TNF Conference took place in Ghent, Belgium (20–23 May 2015).

[144] See, for example, Grewal (2009).

[145] A reference to the work done on *Drosophila melanogaster* (common fruit fly), which Dr Marcos Vidal discusses on pages 59–61.

[146] Balkwill, Ward, and Fiers (1987).

promoting a tumour microenvironment. In the control mice we would see many free-floating clumps of cells, but in the mice treated with TNF they'd develop a very complex tumour microenvironment; there'd be blood vessels, there'd be fibroblasts. TNF was stimulating a tumour microenvironment. So we published a paper in 1989 that I think was labelled 'the paradoxical behaviour of TNF', and there were other bits of evidence at the time countering the fact that TNF was killing cells.[147] There were publications showing malignant cells could make TNF as well, which made some people scratch their heads.

I think it was in 1993 that, using *in situ* hybridization, which was the best tool we had at the time, we showed that malignant cells in human ovarian cancer biopsies were making TNF.[148] Then, the following year, if I remember correctly, we showed that macrophages in breast cancers also made TNF, and this is when the work was going on in RA down at the Kennedy Institute.[149] I can remember going down there a lot and there were so many parallels. Therefore, we began to carry out experiments on the tumour-promoting effects of TNF, particularly in the tumour microenvironment. And then, when George Kollias had the TNF knockout mice, he agreed to send them to us before he published them, and then we did the skin carcinogenesis experiments.[150] And the results were very clear. You could open up a cage and you knew which were the TNF knockout mice because they didn't have a single papilloma. People who were working on the anti-tumour effects of TNF, were, I was told, devastated by the paper we published on this in *Nature Medicine* in 1999.[151]

The other interesting thing was that if you looked at the skin of the mice that are treated with 7,12-dimethylbenz[a]-anthracene (DMBA) and promoted with 12-O-tetradecanoyl-phorbol-13-acetate (TPA),[152] it stimulates a lot of inflammation and you get these papillomas that sometimes progress to malignant tumours. But the TNF knockout mice just didn't get any, and their

[147] Malik *et al.* (1989).

[148] Naylor *et al.* (1993).

[149] Miles *et al.* (1994).

[150] Professor George Kollias is a Member of the Academy of Athens and Professor of Experimental Physiology at the Medical School of the National and Kapodistrian University of Athens.

[151] Moore *et al.* (1999).

[152] The so-called 'initiation/promotion models' of experimental carcinogenesis have been widely used to study skin tumorigenesis. In such models, a topical subcarcinogenic dose of a chemical (DMBA) is first applied to the back of the skin (initiation) followed by topical applications of one or more chemicals (TPA; promotion). For more details, see National Toxicology Program (1996).

skin was incredibly smooth. I've always thought of anti-TNF as a skin cream, you know! I can remember going to Centocor and speaking to some people, and there was a very productive collaboration with Centocor who, I think, were very visionary in those days and really wanted to find other uses for anti-TNF. As you will remember, we were involved in three clinical trials, Phase 1 clinical trials of anti-TNF.

Maini: In ovarian cancer?

Balkwill: Well, there was a Phase 1 all-comers, there was a small study, very much inspired by your work, looking at ascitic disease in end-stage ovarian cancer patients, and just looking to see if we could see changes in inflammatory profile and things like that. Then Martin Gore at the Royal Marsden Hospital did a study in advanced renal cancer, and that was very interesting.[153] There were definitely partial responses in some patients. It's a paper in the *Journal of Clinical Oncology,* 2007.[154] But then Centocor became 'Ortho Biotech Oncology'.[155] I know some of the people who worked at Centocor said they felt they almost climbed to the top of Everest in testing anti-TNF in cancer but they never got to the final point. Now, of course, we all see wonderful things with the immune checkpoint blockade, but both in those trials and with an anti-IL-6 trial that we did in ovarian cancer just a bit later, I do wonder what would have happened if we'd had the modified 'Response Evaluation Criteria In Solid Tumors' (RECIST)[156] – I don't know if you all know this, but the RECIST criteria in Phase 1 or 2 clinical cancer trials are that if a tumour size increases by 25 per cent, then you have to stop the treatment and the patient's disease is said to 'progress'. But once they started immune checkpoint blockade somebody in the States had the courage to say, 'Hang on a minute, maybe the tumour is getting bigger because it's filling with good lymphocytes.'

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[153] Martin Gore is Professor of Cancer Medicine at the Institute of Cancer Research and a Consultant Medical Oncologist at the Royal Marsden Hospital. For more details, see https://www.royalmarsden.nhs.uk/our-consultants-units-and-wards/consultant-directory/professor-martin-gore (accessed 16 March 2016).

[154] Harrison *et al.* (2007).

[155] See note 80.

[156] The RECIST criteria are a set of published rules (published in 2000 and updated in 2009) that define when tumours in cancer patients respond, stabilize, or progress during treatment. For the latest version of these criteria, see Eisenhauer *et al.* (2009).

Walczak: That was Jim Allison telling the clinicians, 'You have to keep on going because they're filling up with lymphocytes, you have to go on!'

Balkwill: And so now there are immune RECIST criteria where if a tumour starts growing a bit then it's not necessarily a sign to stop the treatment, if the patient is feeling well and there are other indications of response. Responses were nowhere near as dramatic as immune checkpoint blockade, but there were certain biologic effects of the anti-TNF and the anti-IL-6, and I was encouraged earlier on because a question for the rheumatologists was there must have always been a worry: how could you give anti-TNF to patients without inducing cancer? Many people said, 'Ah well, this anti-TNF stuff, it causes lymphoma' – that must have been one of the hurdles, you didn't mention that?

Maini: I think it required what we call 'post-registration data', and what has happened is that there are a number of registries in the world now. There's one in the UK, there are very good ones in Sweden, Spain, and the USA, where, basically, patients who are treated with anti-TNF are followed up to see whether the incidence of side-effects is any higher than expected in the general population. What's turning out to be the case in RA is that the incidence of lymphomas is no different, and may even be a bit less, in anti-TNF treated patients than in the RA population not on anti-TNF treatment. That's been very reassuring as there is still a question mark over this issue, because if you look at the data there is some evidence that the very high doses of anti-TNF might be associated with a lymphoma, especially in childhood diseases. So there is some residual concern about the association with an atypical type of lymphoma.

Balkwill: Yes, a gamma delta T cell lymphoma, isn't it?

Maini: I think an alpha beta T cell lymphoma can occur in children, and this has occurred so rapidly after anti-TNF treatment for childhood RA that there may still be a significant risk there. It's not completely clear, but I think the other reassuring thing that's emerging from these registries is that RA itself is a predisposing factor for lymphoma, and there's a 28-fold increase in the incidence of lymphoma in severe RA. So, as patients with less severe disease are being treated with anti-TNF we are not seeing lymphomas now because it's less likely to occur anyway in that population. The story is becoming more clarified, and since anti-TNF is now being used in the minimal possible dose, rather than in massive doses, and when used with methotrexate, it really is possible to titrate a dose of the anti-TNF agents to much lower doses and get prolonged effects.

Walczak: Mentioning the registries it would be interesting to ask given your data, Fran, on the anti-TNF therapies in the pre-clinical models: what has actually happened in the patients who have been suffering from different autoimmune diseases and have been treated with anti-TNF therapy with respect to colon cancer or other inflammation-driven cancers?

Maini: I think data from the British registry and the Swedish registry, I don't want to misquote, and the Germany registry might answer that. I think there's a diminution in the incidence in some of these cancers, right?

Kalden: It's not yet statistically significant but there is a diminution, definitely in nearly all cancers – also in colon cancer – with one exception; that is basaliomas.[157] There is a discussion going on about how solid this finding is. In patients undergoing medication with TNF blockers, all together, none of the registries in Europe have observed a significant increase in malignancy, with the exception of basaliomas, even over a long period of time.

Maini: Which cancer is that?

Kalden: Skin cancer/basaliomas. The mechanism behind the diminution could be the decrease in inflammatory activity but again further data are needed of other malignancies.

Walczak: So the question I asked, of course, is because it would be interesting to see whether there is a reduction in certain inflammation-associated cancer, and, if so, how patients still develop cancer? Under anti-TNF therapy, is their immune infiltrate altered in a way that would make it more likely for a cancer immunotherapy to work? If so, that could suggest that anti-TNF therapy could be combined with anti-programmed cell death protein 1 (PD1)[158] or anti-cytotoxic T lymphocyte-associated protein 4 (CTLA4)[159] therapies in order to enable the right inflammation to happen in the cancer rather than the wrong one. You mentioned previously that prostaglandin expression can be driven by TNF, and, of course, there's also very good evidence that aspirin, which inhibits production of prostaglandins, works very well to prevent, for example, colon cancer. There's a lot of room to look into these things apart from the benefit

[157] Basalioma (or basal-cell carcinoma) is a frequently encountered malignant skin tumour of low metastatic potential.

[158] PD1 is a cell surface receptor that downregulates the activation of T cells and, thus, prevents the expression of autoimmunity.

[159] CTLA4 is a cell surface receptor that prevents the activation of T cells.

achieved in the treatment of autoimmune patients when we look with the right questions at these registries. That way we could already learn a lot about the possible use of TNF blockers in cancer therapy.

Balkwill: I agree. I had a review out in *Nature Reviews Clinical Oncology* two weeks ago discussing the ongoing clinical trials targeting cancer-related inflammation, reviewing the anti-TNF, anti-IL-6, and many other approaches but also suggesting that the way forward is to do the anti-inflammatory treatments and then other immunotherapies that target the adaptive immune system.[160] I think it's something we're certainly working on, and others as well.

Chernajovsky: But the principle by which TNF has worked at the peak of the cytokine cascade has never been shown in cancer, so targeting TNF may not be necessarily the best treatment?

Balkwill: Oh no, I don't think anyone would say that targeting TNF in cancer is the best treatment, but it is a major mediator of cancer-related inflammation, as is IL-6. I think the difference between malignant disease and autoimmune disease in terms of anti-TNF or anti-cytokine treatment is that the autoimmune disease is really more stable, whereas the driver for the abnormal production of the cytokines in a malignancy is the malignant cell itself. It's not the microenvironment, and the malignant cells just overcome the inhibition because they can evolve and escape. If you inhibit with anti-TNF it will make IL-6, or whatever, and I think that's the difference.

Feldmann: There was one clinical trial that was an outlier, which was a six-month anti-TNF trial in chronic obstructive pulmonary disease, and there were four lung cancers that emerged. That's really very interesting because four cancers in six months really suggests that they had started before but they weren't clinically overt. There's now literature that inflammation is also involved in the metastasis process, so, for example, p38 mitogen-activated protein kinase (MAPK) inhibition upregulates metastasis in a number of models of cancer. So it's a very fine balance which we don't understand at the moment.

Maini: I'm just reminded about checkpoint inhibitors for cancer. CTLA4 antibodies and PD1 antibodies induce autoimmunity, and one of the very unpleasant side-effects is the induction of ulcerative colitis type disease, and that has been treated by anti-TNF successfully.

Balkwill: It's a good point, yes, a very good point.

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[160] Crusz and Balkwill (2015).

Saklatvala: I was just going to say that TNF might be a tumour promoter, since it is biologically very similar to phorbol ester, which, of course, is historically probably the oldest tumour promoter and still used experimentally.[161] But presumably you need to know in any particular cancer to what extent the inflammation is promoting the tumour and to what extent that inflammation is TNF-driven, and that's very difficult.

Balkwill: Yes, and it varies from cancer to cancer. I think really we'd like to hear from Marcos now but just to follow up in the context of this meeting, we got a result a couple of weeks ago that I'm now putting into a paper that we're going to submit soon. We were looking at the effects of neoadjuvant chemotherapy in patients with high-grade serous ovarian cancer to see if it changes the immune landscape because then you can go with immunotherapy. To cut a long story short, we did lots of things and there were very interesting effects on T cells, but, of course, I also wanted to look at TNF. When you give a patient the average neoadjuvant chemotherapy, the TNF levels in the plasma, which are much higher than they are in normal people, just go. It doesn't matter if they have a good response or a bad response to neoadjuvant chemotherapy, the TNF levels go right down. But what was absolutely lovely to me is that when we grind up the metastasis from the patient – metastasis from the patient removed at surgery after the chemotherapy – in the plasma, compared with pre-tumour patients, the TNF levels are absolutely rock bottom. And we looked at IL-6 and interleukin-8 (IL-8), and other cytokines, and it's only TNF that really fell after chemotherapy, which really pleased me.

Vidal: To go back to Jeremy's point on tumour-related inflammation or promoting or suppressing tumours, that's work that we attempted to clarify using the fruit fly, *Drosophila melanogaster*, which is a much simpler system. What we found is that TNF signalling perhaps arose in evolution as an epithelial proofreading mechanism. When you have epithelial cells that lose polarity, the apical-basal polarity that they normally have, the haemocytes, which are *Drosophila*'s macrophages, are recruited to that site, they produce *Drosophila* TNF, which in this context is an apoptotic factor. If you block the *Drosophila* macrophage or TNF, the cells that lack polarity will develop into tumours but these tumours will be local, they will not be invasive.

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[161] Phorbol esters act as tumour promoters through activation of protein kinase C. For more details, see Blumberg (1988). The most widely used phorbol ester for the induction of carcinogenesis is TPA; see note 152.

We found that a second hit to the system, in this case the Ras oncoprotein, would allow the malignant cell to hijack TNF coming from the haemocyte, from the macrophage, and then diverted it. Instead of a tumour cell death, this is led into an invasive programme, and it will start producing collagenase and a lot of acting dynamic changes that allow the cell to disseminate. So in that context we have genetically identified which are the components that will dictate whether this kind of inflammatory response will be suppressing or promoting.[162]

Walczak: We found these data highly exciting and very interesting. Of course, we have to remember that in *Drosophila*, the family of TNF molecules is a lot smaller – there's only one – whereas in humans and other mammals, including mice, we have a lot more. So there may be a distribution of the workload between different TNF superfamily members with the different aspects that you've just mentioned. In that respect, it's interesting to note that for two TNF superfamily members – which are very similar to TNF – the FAS ligand[163] and the TNF-related apoptosis-inducing ligand (TRAIL), have been shown, in contrast to TNF, to indeed induce tumour cell death. However, when the cell has begun to be transformed and when you then add further mutations, like *KRAS* mutations[164] – we have basically used the same nomenclature, or the same wording as you, Marcos – the pathway can be hijacked so that now the tumour cell makes use of the CD95/CD95 ligand or the TRAIL/TRAIL receptor system to its own advantage in enhancing the growth and invasiveness of these cancer cells. In this situation, it's also not only good to block TNF but what we've shown is that in some cases it's good to block the CD95 ligand and in others to block TRAIL. In the case of the CD95 ligand, this can be achieved with an antibody to the CD95 ligand or with a CD95-Fc fusion protein, which has, in fact, been tested in patients with glioblastoma multiforme, together with irradiation, and has shown clinical efficacy in these patients.[165] With respect to TRAIL, we've recently shown that inhibiting TRAIL can, in fact, be beneficial in mice – we have used a KRAS-driven model both in the pancreas and in the lung – that in these situations the endogenous TRAIL/TRAIL receptor system is required for cancer cell invasiveness.[166] When we remove the TRAIL receptor

[162] Cordero *et al.* (2010).

[163] See note 86.

[164] *KRAS* mutations are associated with several types of cancer.

[165] See, for example, Wick *et al.* (2014).

[166] von Karstedt *et al.* (2015).

Figure 13: Dr Marcos Vidal (left) and Professor Joachim Kalden (right)

genetically from the cancer cells, the outcome is the same as if we had taken away mutated KRAS. So the entire increase in invasiveness and aggressiveness of the cancer cells endowed by mutated KRAS was neutralized when taking out only the TRAIL system in these cancers. I therefore think that the concept that was first shown for TNF and for which you have then shown very nicely how it actually works in *Drosophila*, Marcos, can be extrapolated into the CD95 system in certain cancers, and into the TRAIL system in others. This means that there is an expansion of the effects that were first observed for the TNF system into at least two additional members of the TNF superfamily.

Vidal: That's very interesting, how conserved these systems are in evolution, and a future challenge that I think will be interesting in the mammalian system is the cell biology of the ligand and the receptor because what is also interesting in *Drosophila* is that the TNF receptor is very focalized at the apical domain of epithelial cells. That perhaps suggests a mechanism by which the loss of polarity will delocalize a receptor and allow them to be recognized by the immunosurveillance cells.

Balkwill: Making the discussion a bit more general, what does everyone think the main role of TNF is, whether it's in the fly, the mouse: why do we have TNF?

Feldmann: It's a fire alarm.

Balkwill: Ah, you think it's a fire alarm?

Feldmann: It's the quickest cytokine to be produced in any injury, so whether it's ultraviolet light, burns, immunity, you get a rapid induction of TNF, which coordinates leukocyte recruitment, so it's a fire alarm.

Kalden: If you look at the phenotype of a TNF knockout, I think they are living quite a nice life.

Walczak: Well, if these mice had to live on the London tube they probably wouldn't anymore because we're endemic for certain diseases there that they would die of. We have to look at it from an infectious disease perspective and obviously without TNF you have a lot of problems with facing life when you're exposed to everything that our predecessors in evolution have been exposed to.

Balkwill: Do you think it's a possibility – this is my own pet theory – that TNF might be a fire alarm in the young, and maybe even the middle aged, but in old age it's a grim reaper?

Williams: I think it's a fire alarm but I also think that it's a feedback mechanism, and that, like many pro-inflammatory mediators – prostaglandin E_2, for example – they induce localized inflammation, then feed back and suppress the immune response, suppress T cell responses. I think that TNF induces inflammation but then feeds back on the immune system to dampen down the immune response, which is driving the whole process.

Chernajovsky: Are my memories right or am I getting really old: the knockout mice, they didn't have proper lymphoid organs?[167] The structure was not right.

Balkwill: Yes.

Chernajovsky: It was just lymphotoxin? In lymphoid organogenesis they are very important, not just as a mediator of inflammation in adults.

Maini: There is a researcher at McMaster University called Dawn Bowdish.[168] Dawn worked in Oxford before, she's a Canadian, and she's back in Canada

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[167] Professor Chernajovsky noted: 'TNFα knockout mice completely lack splenic primary B cell follicles and cannot form organized follicular dendritic cell networks and germinal centers.' E-mail to Dr Apostolos Zarros, 25 March 2016. For more details, see Pasparakis *et al*. (1996).

[168] Dr Dawn Bowdish is Associate Professor at the Department of Pathology and Molecular Medicine of the McMaster Immunology Research Centre, Hamilton, Ontario, Canada. For more details on her research activity, see http://fhs.mcmaster.ca/pathology/contact_us/faculty/faculty_bios/bowdish.html (accessed 16 March 2016).

now. When I was visiting her laboratory recently, she showed me some very interesting data on TNF knockouts. Basically, it is an anti-ageing phenotype. So she has what she calls 'TNF sleek' mice, or 'TNF knockout sleek' mice.

Balkwill: Yes, it's just like the skin cream because we used to say the skin of the TNF knockout mice was as smooth as a baby's bottom – that was the term that came from the animal house. The TNF knockout mice were indeed 'sleek' mice.

Maini: I don't want to misquote her because I haven't seen her work in print, and I don't think the anti-ageing thing is published yet. I think she shows some quite important lack of a protection, in these knockout mice, that there wasn't any undue susceptibility to certain infections, which is surprising for me. We know that tuberculosis infections are increased, so intracellular infections are increased, but it was by no means established that extracellular bacteria infections are increased in these patients.

Balkwill: There was also that paper on the brain, I think in *Nature*, on sensing aging in the brain and TNF produced in the brain.[169]

Tansey: I just wonder whether I could ask you to reflect, in the final half hour, on some broader historical themes. It's absolutely fascinating, this scientific discussion, but we are here to discuss the history of TNF and we want to try and get behind the papers, the published papers, and I wondered if I could ask you, these may be irrelevant questions but I don't think they are: what kind of networks were important; what kind of societies and clubs; where did you talk about your papers; how did you all get to know each other? Because this is a new field, this is not necessarily the Physiological Society nor the Biochemical Society, for example. Who funded this work? We've heard about some funding, and of particular companies: where was the MRC or the Wellcome Trust, or any of these kinds of organizations? And institutions? We've heard quite a lot about the Kennedy Institute, and some companies. Were they unique? Were they the only organizations? We've heard a little about opposition: again, may I ask if you could just reflect and perhaps say something on those issues?

Walczak: Tiny and Joachim, you both mentioned the weekend you spent together in your house discussing…

Kalden: Only one weekend.

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[169] Zhang *et al.* (2013).

Walczak: I know, but, I think that would be important to mention in this context, right? These personal connections are important in all walks of life, including science.

Kalden: I think it is always important, and this is reflected, for example, by the German Research Foundation.[170] They are putting money into networks in, say, B cell physiology, pathophysiology, getting different good centres together, even some centres working in Austria or in France. Bavaria has established an immune-therapeutic network where all five university hospitals are included. New therapeutic pathways will be explored using cell therapeutic approaches as well as different antibody constructs and small molecules. It is hoped that these networks will get into a fruitful cooperation with the pharmaceutical industry. Industry should be interested specifically in financing clinical trials. Neither the German Research Foundation nor the Ministry of Science and Technology really have the financial situation to finance huge clinical trials. It would be interesting to see how the interaction between university institutions and respective pharmaceutical industries are going in other countries.

Balkwill: But back then, as we were talking about the 1980s, it was really the company-sponsored money that funded the clinical trials?

Feldmann: I think the role of companies is both a plus and a minus. Sometimes it's positive, but at the moment the willingness of companies to invest in anything that looks like research is not too high. But to go back to Tilli's question: what were the important networks? Well, I mentioned already setting up the cytokine meetings, so Jo Oppenheim was very important.[171] There are cytokine societies that have struggled to survive that end up merging between the Cytokine Society and the Interferon Society – one survivor was better than two lame ones.[172] The TNF meetings have been very important in driving the field, and I think part of my contrary view to the role of industry is based on having run one of these meetings, as did Henning,[173] yet the Pharma companies

[170] The Deutsche Forschungsgemeinschaft; see website at http://www.dfg.de/en/ (accessed 16 March 2016).

[171] For Dr Joost (Jo) J. Oppenheim, see note 59.

[172] The 'International Cytokine and Interferon Society' was a merger of the 'International Cytokine Society' and the 'International Society for Cytokine and Interferon Research' in 1987; see http://cytokines-interferons.org/About-ICIS/Constitution-and-Bylaws/ICIS_ByLaws_Final.aspx (accessed 22 March 2016).

[173] Professor Henning Walczak commented: 'the series started with the TNF meeting in 1987 in Heidelberg […] the latest one (the 15th in the series) was organised by myself and Peter Vandenabeele in Ghent, Belgium, in 2015, and the next one in this biannual series will take place in Singapore in the spring of 2017.' E-mail to Dr Apostolos Zarros, 27 May 2016.

are selling almost 30 billion dollars of anti-TNF but they will give you nothing to support the meetings from where the material that led to these sales came. The companies will not give you the value of a single patient's therapy to help feedback into the academic base.

[Unidentified participant]: It's a little bit different with the 'Advances in Targeted Therapies';[174] that is a meeting we are running now for 20 years, and it is both inclusive of basic and clinical science.

Feldmann: We could argue the academic networks are important but I think it's the informal ones that matter, and they are driven through relatively small meetings where you can find each other. But the biotech industry in its first life was very much more academic. They were really academics who had somehow raided the bank: Genentech, Immunex, Amgen, and others, were behaving like super universities doing exceptionally good research; they cloned most of the cytokines, and shared the reagents generously. This is no longer the case.

Balkwill: I would agree with that; I think the biotech industry was very instrumental in research. In the UK, I think also interactions between cancer researchers and those working on infections and inflammatory diseases were important. Do you remember the British Cytokine Group (BCG) that Fionula Brennan and I ran for a long while?[175] Audiences were about 100 people and maybe one international speaker, and that was really good. That came out of the TNF meeting in Heidelberg in 1987,[176] where I realized that there were a lot of people working on TNF in other diseases, not just cancer, and that was really useful for quite a few people. It sort of faded after a while.

Kalden: It was a meeting which was organized by Daniela Maennel and colleagues, if my memory serves me correctly.

Balkwill: Yes, that was the one in Heidelberg. But there were two meetings in Heidelberg. There was a very early one.

Kalden: I believe one of the organizers was Holger Kirchner.

Balkwill: The TNF meetings, I agree, but I think in the UK the BCG was useful; we ran it for about ten years, if not longer. I would say also organizations

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[174] The proceedings of the 'International Symposium on Advances in Targeted Therapies' (I–XIV) are published as Supplements to the *Annals of the Rheumatic Diseases* (1999–2013).

[175] For more details, see Balkwill and Burke (1989).

[176] Bonavida *et al.* (1988).

like Cancer Research UK that renewed programme grants, and presumably the Arthritis and Rheumatism Council was another one.[177]

Maini: So, Tilli, if I go back to focusing on the anti-TNF story and the funding: the funding came from multiple sources, and I think Marc and I were faced with challenges throughout. I think all the researchers were quite inventive in raising money and diverting money in various directions that weren't necessarily intended by the person giving you the money. So there's no doubt that the Kennedy Institute funding was by the Arthritis and Rheumatism Council, now known as Arthritis Research UK. They gave money – I was the Director of this institution. It allowed me to recruit Marc and his group into the funding stream; that helped. But, actually, when it came to the crunch, the referees to whom we submitted our anti-TNF story weren't enthusiastic about it and the funding had to come mainly from our industrial grants, but also from Fellows who came to work with us with their own funding. For example, there was an Australian chap called Mike Elliott who came to work with Marc and me from Melbourne, from the Walter and Eliza Hall Institute[178]. He had worked with the haematologist Don Metcalf there, but wanted to do clinical work, and although he had never done any clinical trials in his life, we involved him in the trials because he's highly intelligent. It was very useful having a very good, motivated, hardworking Aussie in our group who could actually be present for recruiting and managing the patients for our initial trials. That funding came from the Australian Rheumatism Association or the Australian MRC; perhaps both of those. The Nuffield Foundation gave us some money for some minor part of our work, a small project grant, I think. We had several little grants that were being put into the kitty, ultimately leading to these big trials, which were entirely funded by Centocor.

Kalden: Could I just make clear what I was saying: funding of research in Germany is really fantastic. This is why research is developing very well, both in basic- and more clinical-related activities. In this respect, it is worth mentioning that basic science is nowadays also performed in clinical institutions, so we are coming up with data from basic institutes in clinical as well as in basic science. The question is, really, which institutions will take up this data for further investigations? In order to translate some of these data into clinical practice, I believe that the help of pharmaceutical companies is needed.

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[177] See note 11.

[178] See note 24.

Walczak: I would like to touch upon a different point with respect to the history of modern biomedicine and the role that the companies like Genentech and Immunex have played, at least in the field we have been talking about – we mentioned these names throughout. They could exist at the time when they were founded because they were experiments for venture capitalists that had worked. In the meantime, many other experiments that were supposed to be based on the Genentech and Immunex principle, and Biogen and DNAX for that matter, have been carried out, and there were only a few additional ones that were really as successful as Genentech, Immunex, and Amgen, so these experiments, these few experiments from the venture capitalist standpoint, have worked. That's why there was this 'playground', as Steve Gillis, the founder and first Chief Executive Officer of Immunex, used to call it; even though he was probably also behind one of the things that was mentioned here before. I joined Immunex after he had left, but still benefitted from the 'playground' he had left behind. You could see how it worked, that there was this group of, I would say, top scientists in the field of immunology at the time that were gathered on a theme, and they would work without having to worry about grant money because no one there was worried about any grant, simply because you didn't need grant money. There was enough money to do anything you would like to do; we came in as postdocs and we could do what we wanted to do. There was no restriction whatsoever on what we wanted to do, on what we could have done. It's important to mention that this type of biotech industry does not exist anymore today. The reason is that in the investment banks of today where mathematicians analyse the pharmaceutical industry and the biotech firms, the people who are making the calculations think that the entire biotech concept hasn't worked. I would counter argue that the concept has worked but that you need the right brains behind these concepts.

Genentech and Immunex were very different from many of the other companies because they did not have a high degree of mediocrity in these institutions. They had very many of the world's leading scientists in their respective fields. I still believe that the concept of putting roughly 50 million euros, dollars, or pounds a year into the best science in a particular field will be able to yield something similar to what turned out to be the 10 billion that Amgen paid for Immunex 16 years after it was founded. I am convinced that concept would still work but you need to get the right people into this concept, on both the scientific and investment side. Unfortunately, the people who come up with all the important questions that decide on whether an investment will happen or not have taken

into account too many of the mediocre companies that didn't work, so they say the concept hasn't worked. But I argue that it does work, you just need the right quality and critical mass of excellence.

Chernajovsky: What I want to say is that all the work on anti-TNF has been exemplary for many other disciplines in medicine, and it's not just Fran who has been looking at the work of Tiny and Marc with admiration and trying to follow it up from the cancer perspective. The people who work with MS have done the same, and all the biological registries that were established to follow up on the patients who were treated with anti-TNF and biologicals were exemplary to many other disciplines. This story is not only about the success of the anti-TNF trials, it's the success of how to approach a medical problem and to deal with it with the most modern medicine that you can. The fact that, as of course as we know, times have changed and there were a lot of good things happening in the 1980s, and late 1970s, because if you cloned a gene a company would be knocking on the door of the lab asking, 'Hey, can we have it?' That doesn't happen anymore. So things have changed a lot but all this work has been absolutely instrumental to show how to progress a project in medicine.

Maini: I just want to comment that our work at the Kennedy Institute was unusual in that we were able to take it from the very beginning to the very end, to registration.[179] Now, there are many reasons why this became possible, but we were involved at every stage of it, including the Phase 3 trials. I think that a lot of work in translation gets stuck at some stage or another because there isn't the continuity of input. That's a serious problem. You can see, for example, that companies are producing lots of new molecules, including therapeutic molecules and targets, and academics are producing quite interesting information on pathogenesis and possible candidate molecular targets, but it's sort of getting stuck somewhere along the line of development up to about Phase 1. After that, there isn't the input or the insight, or collaboration, of the sort that is needed between scientists and clinicians and industry to actually talk and work without barriers. Many of us who get asked to talk to industry, for example, will find that you go and talk to them, and you never know what happened to that conversation. It kind of disappears into the ether, there's no feedback, and there's a lot of confidentiality involved with it. And so, it was the age of innocence that Marc and I enjoyed where we could actually talk freely about what we did, without thinking too much, frankly to our detriment, because we could have patented some of the stuff that we did, which we didn't patent.

[179] Feldmann and Maini (2003).

That kind of culture, sadly, has also been asphyxiated by the imposition of bureaucratic structures, and I personally think that these networks that are being set up by, for example, the National Institutes of Health (NIH), by Germany, and in the UK, aren't going to work because they don't have the chemistry of the individuals involved. They are going to be completely sterile. There is an immune network in the USA that we know has had quite a lot of NIH money, but it's got absolutely nowhere. They have done some trials in insulin-dependent diabetes mellitus with anti-CD3. They saw the expected Epstein-Barr virus infections and so didn't go very far, they didn't produce anything very novel, and I think that network has folded.

Feldmann: It's still going but it hasn't produced anything. I think if we're talking about facilitators and inhibitors of progress, I've alluded to the age of innocence when there were no MTAs, but, actually, I think in British science – and I don't think it's any better in America where I spend a bit of time, and I don't know about Germany – the fixation of universities and other organizations on trying to file patents to cover the miracle of some discovery, of making money, is a real impediment to science.

When I was at Imperial College and our Institute was getting significant royalties and I was involved in management, we got little memos of how much the Imperial tech transfer office generated in cash. So in a good year they generated three million pounds. You can guess what they cost. Every year they ran at a loss. But the loss to scientific productivity has been monumental because every time you wanted to borrow somebody's reagent it would take you six months, and probably meant that at least half the things that you wanted to do got stopped.

In the history of modern biomedicine, a very interesting topic to focus four hours on might be the patenting system: the pros and cons. Is the licensing system fit for purpose? I don't think it is.

Having been involved in one of the few discoveries that's made a lot of money, we know how many there are: in the UK it's less than one every ten years. So for the lottery effect, we have this enormous albatross that academia is carrying. Now I could go on and on, but I think I've said enough. I think if we're interested in what's funded and what's hindered, then I think at the moment we have a very strongly negative delusion about the capacity of academic research to find things worth patenting. And the NHS is even worse; the NHS set up offices to patent what it does. I've had visits from these people, but I'd better not say anymore.

Balkwill: Well, the wine is ready so closing comments maybe slightly on a more positive note? [Laughter]

Woody: Could I make a quick comment?

Balkwill: Oh please, Jim! We'd love to hear from you.

Woody: My congratulations to all the people who have helped allow the larger anti-TNF story to emerge. We have to take some pride in the fact that, as Marc has pointed out, there are no patients with RA left in wheelchairs because of this class of drugs. It's rare to have a clinical breakthrough of that gravity, so my congratulations to everyone on this list who participated; it was a great achievement. Thank you.

Balkwill: Thank you very much, and thank you for being there all the time. Is there anything else, Jim? We've not really given you too much of a voice, and yet I think your part was very critical. Is there anything else that you want to say before we finish? We can't offer you a glass of wine, sadly.

Woody: We also had other trials; we looked at TNF distribution and found that it was distributed in a lot of different tissues. As I noted earlier with the anti-CD4 trials, we stopped them as they were clinically ineffective. We tried anti-TNF in MS, which actually made the patients worse, which was an issue when Roche/Genentech treated a number of MS patients with lenercept[180] – it actually made quite a few patients worse. It is unclear why that happened. So we did a lot of other things, but the fundamental discovery that our anti-TNF was clinically beneficial to patients with RA, Crohn's disease, and psoriasis were the pivotal achievements of this whole project.

Tansey: Well, that's been a fascinating afternoon, thank you all very much indeed. I'm actually a former neuroscientist, and some of the things you were talking about reminded me of the 1980s when every time one picked up a fresh issue of *Trends in Neuroscience* there was a new molecule that was bioactive, there was a new receptor that had been found, and we had very good relationships with pharmaceutical companies in those days. What was very important was a point that was made early on: pharmaceutical companies made the tools of the trade for us; they made the drugs that we could then use for further experiments. That was very, very important.

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[180] See note 108.

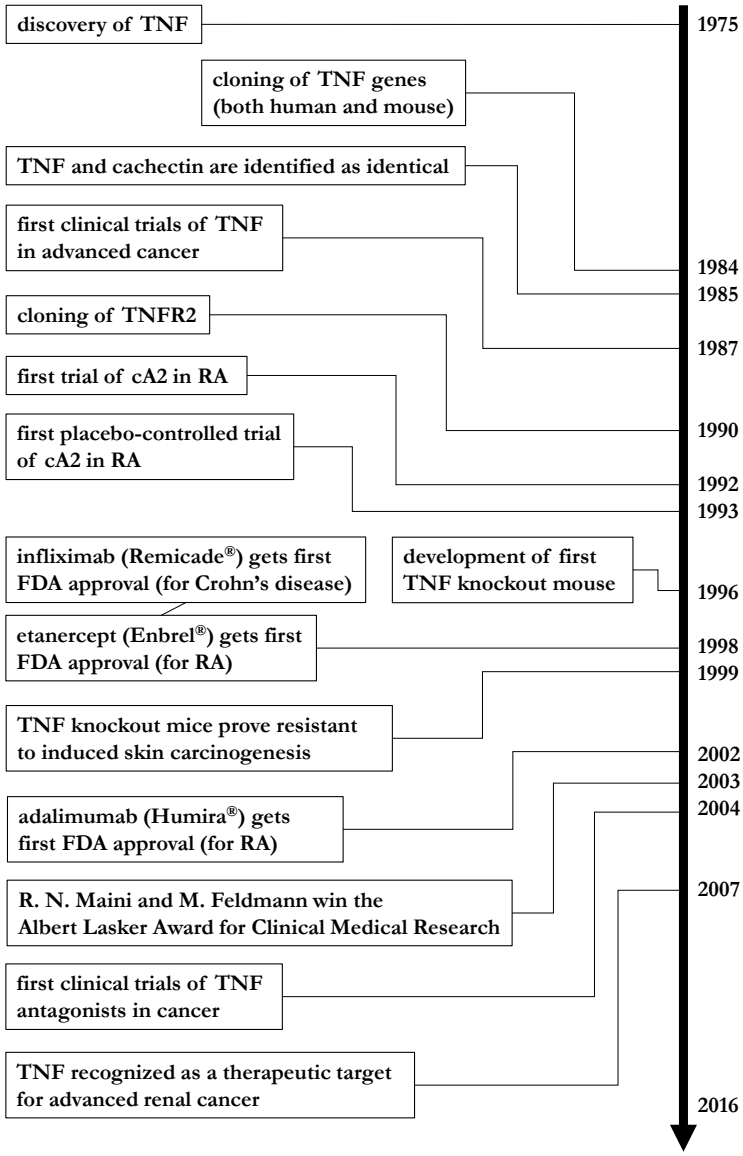
You might be surprised, or perhaps not surprised, to learn that some of the comments that came at the end of this meeting are very common at some of these meetings.

I'm particularly grateful to Fran, of course, for chairing the meeting, participating and being one of the people who suggested it in the first place. So thank you very much, Fran.

Balkwill: Well, I did nothing, it was Tilli's team. I've thoroughly enjoyed it; I'm just so glad that the seminar finally happened.

Appendix 1

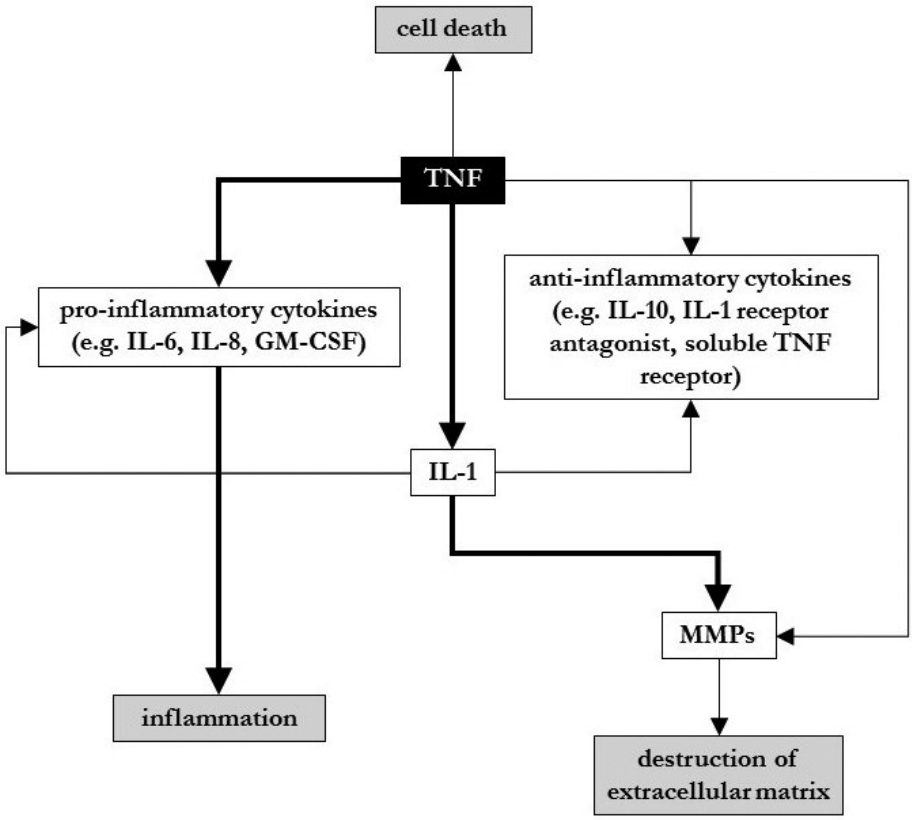
Timeline of important events in the history of TNF[181]



[181] Illustration devised by Dr Apostolos Zarros based on data from: Balkwill (2009) and Dayer (2002).

Appendix 2

Simplified overview of the main biological actions of TNF in rheumatoid arthritis[182]



[182] Illustration devised by Dr Apostolos Zarros based on data from: Feldmann (2009) and Dayer (2002).

Appendix 3

Overview of TNF inhibitors mentioned in the current Witness Seminar transcript[183]

Drug	Trade name (current company)	Description and indications (with dates of FDA approval)
Infliximab	Remicade® (Janssen Biotech)	chimeric (mouse and human) anti-TNF monoclonal antibody Crohn's disease (1998), RA (1999), ankylosing spondylitis (2004), ulcerative colitis (2005), psoriatic arthritis (2005), psoriasis (2006)
Adalimumab	Humira® (AbbVie)	human anti-TNF monoclonal antibody RA (2002), psoriatic arthritis (2005), ankylosing spondylitis (2006), Crohn's disease (2007), psoriasis (2008), juvenile idiopathic arthritis (2008), ulcerative colitis (2012), hidradenitis suppurativa (2015)
Etanercept	Enbrel® (Amgen/Pfizer)	fusion protein that fuses TNFR2 to the constant end (Fc) of an IgG1 antibody RA (1998), juvenile idiopathic arthritis (1999), psoriatic arthritis (2002), ankylosing spondylitis (2003), psoriasis (2004)

[183] Table devised by Dr Apostolos Zarros based on data from: Shealy and Visvanathan (2008) and Kalliolias and Ivashkiv (2016).

Glossary

The following textual and web-based sources were consulted: *Churchill's Illustrated Medical Dictionary* (1989). New York: Churchill Livingstone; *Roitt's Essential Immunology* (12th edition) (2011). West Sussex: Wiley-Blackwell; *Immunology: A Short Course* (5th edition). Hoboken, NJ: John Wiley & Sons; *Merriam-Webster* (online dictionary), http://www.merriam-webster.com/

Adaptive immune disease
Disease resulting from dysfunction of the adaptive (or acquired) immune system; an autoimmune disease.

Antibody
Protein of high molecular weight that is synthesized by B cells following stimulation by an antigen in order to react specifically against the latter; immunoglobulin.

Autoimmune disease
Disease characterized by autoimmunity.

Autoimmunity
Pathophysiological state caused by a cell-mediated or humoral immunological response to antigens of one's own body.

B cells
Type of lymphocytes that bear antigen-binding antibodies on their cell surface, have antibody secreting properties, and/or secrete cytokines. B cells mature in the bone marrow. Also known as B lymphocytes.

Basalioma
Frequently encountered malignant skin tumour of low metastatic potential; basal-cell carcinoma.

Chimeric antibody
Synthetic (recombinant) antibody that is actually a fusion protein, created through the joining of two or more genes that originally coded for different proteins.

Clone
Aggregate of genetically identical cells or organisms produced by a single progenitor cell or organism.

Cluster of differentiation (antigen nomenclature)
Protocol for the identification and the classification of antigens. It could refer to either cell surface receptors or ligands.

Combination therapy
The use of more than one therapeutic approach/medication to treat a certain disease.

Crohn's disease
Chronic disease characterized by transmural inflammation and deep linear ulceration of the distal portion of the ileum (small intestine) and/or of the colon.

Cytokine
Class of proteins that modulate the function of immune cells; immunoregulatory proteins.

Endotoxin
Pathogenic lipopolysaccharide of the outer membrane of Gram-negative bacteria, which can be released upon cell lysis.

Fc
The crystallizable, non-antigen-binding fragment of an immunoglobulin (antibody); fragment crystallizable region.

Fusion protein
The product of a fusion gene that is created through the joining of two or more genes that originally coded for different proteins; chimeric protein.

Helper T cell
Subclass of T cells that recognize foreign antigens and secrete cytokines that activate other T cells and induce B cell proliferation. A key category of immune cells in regulating immune responses. Also known as helper cell or T helper cell.

Humanized antibody
Genetically engineered monoclonal antibody of non-human (usually murine) origin in which all but the antigen-binding complementarity determining regions' sequences have been replaced with sequences derived from human antibodies. These antibodies cause lower immunogenicity.

Immunogenicity
Ability to induce an immune response.

Immunosuppressive
Ability to suppress natural immune responses.

Inflammation
Response to injury caused by physical, chemical, or biological causes. It serves as a mechanism of dilution, containment, and destruction of the injurious agent. Clinically characterized by redness, swelling, pain, heat, and loss of function.

Innate immune disease
Disease resulting from dysfunction of the innate immune system; an autoimmune disease. Not to be confused with 'adaptive immune disease'.

Interferons
Group of cytokines produced by immune cells in response to several pathogens (particularly viruses) and tumour cells. They play a key role in immune response regulation.

Interleukins
Leukocyte-secreted cytokine class involved in immunoregulation.

Knockout
Term used to describe animals that are generated through genetic engineering and have a specific gene deliberately made non-functional. The replacement of a functional gene with a defective (non-functional) copy of the gene is an approach used to define *in vivo* the function of a specific gene's product.

Leukocytes
White blood cells.

Ligand
Term used to define a molecule recognized by (at least) one binding molecular structure, such as a receptor.

Lymphocytes
Class of leukocytes that are typical cellular elements of the lymph and that include major cellular subclasses of the immune system, such as the B and the T cells.

Lymphoma
Class of tumours that arise from lymphatic cells. The two main categories of lymphomas are Hodgkin and non-Hodgkin lymphomas.

Macrophages
Class of leukocytes that undertake phagocytosis to destroy foreign antigens and serve as antigen-presenting cells. They derive from monocytes and can be found in all tissues.

Monoclonal antibodies
Antibodies deriving from a single B cell clone, thus being of identical antigen-binding sites and immunoglobulin isotype.

Monocytes
Class of leukocytes that are characterized by a large cytoplasm containing a large, smooth, well-defined, indented, slightly folded, oval, kidney-shaped, or notched nucleus. They can differentiate into macrophages and other leukocyte classes.

Mutation
Process of undergoing a permanent change in the hereditary material of an organism, that could affect the functionality of a gene product and the organism's phenotype.

Placebo-controlled trial
Trial (clinical study) in which a group of subjects receives the tested treatment, and a separate control group receives a sham treatment without intrinsic therapeutic value, but administered as if it were a therapy, i.e. a placebo.

Pre-clinical models
Term referring to *in vitro* and *in vivo* experimental approaches to diseases, taking place as part of an efficacy and safety evaluation of a drug, prior to its testing on humans. If specifically used for *in vivo* experiments, the term reflects the appropriate experimental setting (either through genetic modification and/or disease induction) in which an animal can be forced to undergo a state that closely simulates a (human) disease or a representative aspect of it.

Prostaglandins
Unsaturated fatty acids that are formed as cyclooxygenase metabolites, are composed of a chain of 20 carbon atoms, and perform a variety of biological actions (including the increase of vascular permeability, the mediation of fever, and the modulation of immunological responses).

Psoriasis
Chronic disease of the skin characterized by well-demarcated, red, itchy patches, covered with white/silvery scales.

Recombinant (protein)
Protein produced by genetic engineering.

Regulatory T cells
Subclass of T cells that regulates or suppresses immune responses. A key category of immune cells in preventing autoimmunity.

Rheumatoid arthritis
Chronic autoimmune disease that affects the joints. It is clinically characterized by inflammation, swelling, stiffness, pain, and (even) destruction of joints.

Synovium
Anatomical term for the inner layer of the articular capsule of the synovial joints. It secretes the synovial fluid. Also known as synovial membrane.

T cells
Type of lymphocytes that bear highly specific cell-surface antigen receptors and regulate cell-mediated and humoral immunity. They mature in the thymus. Also known as T-lymphocytes.

Biographical notes*

Professor Fran Balkwill

OBE PhD HonDSc FMedSci (b. 1952) studied Cellular Pathology at the University of Bristol, and undertook her PhD on leukaemia cell biology in the Medical Oncology Department at St Bartholomew's Hospital under the late Gordon Hamilton-Fairley. Her postdoctoral research was carried out in Joyce Taylor-Papadimitriou's laboratory at ICRF Lincoln's Inn Fields (now the Cancer Research UK London Research Institute), where she began to study interferons and their potential as cancer therapies. She is now Professor of Cancer Biology at Barts Cancer Institute, Queen Mary University of London (QMUL), where she leads the Centre for Cancer and Inflammation. She is also Co-Research Lead for Queen Mary's Institute of Bioengineering. In 2006 she was made a Fellow of the Academy of Medical Sciences and has served on its Council. Fran is also actively involved in communication of science to non-specialist audiences, especially young people. Fran is Director of the Centre of the Cell, a biomedical science centre for children, educational website, and outreach project in East London. She is also a non-parliamentary board member of the Parliamentary Office of Science and Technology, Chair of Understanding Animal Research and serves on MRC and European Research Council (ERC) grant committees. Fran was awarded an OBE in the 2008 Queen's Birthday Honours list. In 2015 she was awarded the honorary degree of Doctor of Science *honoris causa* by the University of Bristol.

Professor Fionula Brennan

BSc PhD (1957–2012) completed her BSc and PhD in immunology in Bristol. From 1989 she worked with Ravinder Maini and then Marc Feldmann, progressing to a Professorship in 2001. She was very involved in cytokine research, co-leading the 'British Cytokine Group' with Frances Balkwill. She was a key participant in the discovery of TNF as a therapeutic target in RA, and continued to contribute to this field.

Professor Yuti Chernajovsky

PhD FRCP (b. 1954) studied biology at the Ben Gurion University in Beer Sheva, Israel

* Contributors are asked to supply details; other entries are compiled from conventional biographical sources.

(1976), followed by studies in molecular biology at the Weizmann Institute of Science Rehovot, Israel. He obtained an MSc in 1978 and PhD in 1983, during which period he cloned and expressed several interferon genes. His postdoctoral studies were at the ICRF in London, and in 1987 he joined the faculty of the Immunology Department at MD Anderson, where he developed gene therapies for cancer. At the end of 1990 he joined the Kennedy Institute of Rheumatology in London where he continued to work on gene therapy for autoimmune diseases. In 1999 he was made Arthritis Research UK Chair of Rheumatology at Barts and The London School of Medicine. He retired in 2014 and currently serves as consultant to a biotech company he founded, Stealthyx Therapeutics Ltd. He is Emeritus Professor of Molecular Medicine at QMUL.

Professor Jon Cohen

MSc FRCP FRCPE FRCPath FFICM FMedSci graduated from Charing Cross Medical School in 1974 and after house officer appointments went to Hammersmith Hospital & the Royal Postgraduate Medical School to begin training in academic medicine. He worked initially for Professor Sir Keith Peters in nephrology, but soon recognized that his interests lay in infection and particularly in opportunistic infection in immunocompromised patients. At that time a training in academic infectious diseases was not available in the UK, so he was successful in obtaining a Wellcome Trust fellowship to first obtain an MSc in microbiology at the London School of Hygiene & Tropical Medicine and then to move to work with Professor David Durack in the Infectious Diseases Department of Duke University in North Carolina. Returning to Hammersmith, he set up the Department of Infectious Diseases, and eventually became Chairman of the joint Department of Clinical Microbiology & Infectious Diseases. In 2002 he was appointed as the Foundation Dean of the new Brighton & Sussex Medical School, eventually stepping down in 2013 to take on the role of President of the International Society for Infectious Diseases. His main research interests have been in sepsis and septic shock, and he has published widely on both basic aspects of pathophysiology as well as leading major international clinical trials of new therapies.

Professor Charles Dinarello

MD (b. 1943) is Professor of Medicine and Immunology at the University of Colorado School of Medicine, and Professor of

Experimental Medicine at Radboud University in the Netherlands. Professor Dinarello received his medical degree from Yale University and clinical training at Massachusetts General Hospital. From 1971–1977, he was an investigator at the National Institutes of Health in Bethesda. In 1998, he was elected to the United States National Academy of Sciences, and in 2011, he became a Foreign Member of the Royal Netherlands Academy of Arts and Sciences. He is a member of the Board of Governors of the Weizmann Institute (Israel) and Ben Gurion University (Israel), former Vice President of the American Society of Clinical Investigation, and was President of the International Cytokine Society. He has received honarary degrees from the University of Marseille (France), the Weizmann Institute of Science (Israel), the University of Frankfurt (Germany), Roosevelt University (USA), Albany Medical College (USA), Radboud University (Netherlands), and Trinity College (Ireland). For his contributions to the field of cytokines and medicine, he received the Squibb Award (USA), Ernst Jung Prize in Medicine (Germany), Chirone Prize (Italian National Academy of Medicine), Carol Nachman Prize (Germany), Sheikh Hamdan bin Rashdid al Madktoum Award (United Arab Emirates), Beering Prize (USA), Albany Prize in Medical Research (USA), Crafoord Prize of the Royal Swedish Academy of Sciences (Sweden), Paul Ehrlich Prize (Germany), Bonfils-Stanton Prize (USA), the Novartis Prize in Clinical Immunology (Switzerland), and the Bonazinga Award (USA).

Professor Sir Marc Feldmann

Kt AC FMedSci FRS (b. 1944) graduated in medicine at the University of Melbourne, before taking a PhD at the Walter & Eliza Hall Institute studying *in vitro* immune responses and immune regulation with Sir Gustav Nossal. He undertook postdoctoral research (1972) with Avrion Mitchison at the ICRF Tumour Immunology Unit, which led to the generation (1983) of a new hypothesis for the mechanism of autoimmunity. Testing this idea led him to leave ICRF and move to the Kennedy Institute of Rheumatology. There, with Maini and Brennan, TNF was defined systematically as a therapeutic target for RA, and, together with Maini, he led clinical trials to verify this concept in patients. This work has led to much recognition, election to Academies of Science in Australia, USA, and the UK (Royal Society and Academy of Medical Sciences), as well as to major research prizes

shared with Ravinder Maini, including the Crafoord Prize of the Royal Swedish Academy (2000), the Albert Lasker Clinical Research Award (2003), the Paul Janssen Prize (2008), the Ernst Schering Prize (2010), and the Canada Gairdner Award (2014). Feldmann was also 'European Inventor of the Year' in the 'Lifetime Achievement' category in 2007. Knighted in 2010, he also received the Australian equivalent 'Companion of the Order of Australia' in 2014.

Professor Walter Fiers

PhD (b. 1931) obtained a degree of Engineer for Chemistry and Agricultural Industries at the University of Ghent (1954) and then started his research career as an enzymologist in the laboratory of Laurent Vandendriessche in Ghent. He then worked in Copenhagen before obtaining a fellowship from the Rockefeller Foundation and joining molecular biologist Bob Sinsheimer's group at the California Institute of Technology as a postdoc in 1960. In 1962, he moved to Madison, Wisconsin, to work in the laboratory of future Nobel laureate, Gobind Khorana. At the end of 1962, Dr Fiers set up the Laboratory of Molecular Biology at the University of Ghent. His research led to his participation in the development of a new discipline that later evolved into 'recombinant DNA technology'. Dr Fiers and his colleagues managed in 1980 to clone and express the gene coding for human IFN-β and, later, of IL-2, IFN-γ, and TNF. In 1997 he retired and became Professor Emeritus, and the following year he retired from his position as Director of the Laboratory.

Professor Joachim Kalden

MD PD (b. 1937) studied medicine at the University of Tübingen. He was a research fellow at the Department of Therapeutics and the MRCs Clinical Endocrinology Unit at the University of Edinburgh from 1967 to 1970. He undertook his habilitation at the Department of Internal Medicine, Hannover Medical School, where he was also an assistant in the Department for Internal Medicine, and became a *Privatdozent* in 1973. From 1974 to 1976 he was Consultant Physician in the Department for Internal Medicine, Clinical Immunology and Rheumatology at Hannover. In 1977 he was appointed Director of the Department of Internal Medicine III and Institute for Clinical Immunology, in which post he remained until his retirement in 2006, then becoming Director Emeritus. His major research interests over the years have been in the pathogenesis of rheumatic diseases, with emphasis

on SLE and RA, including the development of new therapeutic principles. He was President of EULAR from 2001 to 2003, and he has been an active member of many national and international scientific boards and committees (including the Chairmanship of the EULAR Standing Committee for Clinical Trials) and has served as Chairman of the World Health Organization/International Union of Immunological Societies Standardization Committee. He was a Member of the Senate of the German Academy of Science and he is also a Member of the Bavarian Academy of Science. He was awarded the honorary degree of Doctor of Medicine *honoris causa* by the University of Berlin in 1998, the University of Lund in 2005, and by the Hannover Medical School in 2013. Among numerous awards are the International Rheumatology Award of the Japanese College for Rheumatology in 2005 and the AESKU prize for Life Contribution to Autoimmunity, Sorrento, Italy in 2006. He has published more than 600 articles.

Professor Sir Ravinder Nath (Tiny) Maini

Kt FRS FMedSci FRCP MB BChir (b. 1937) studied medicine at Cambridge University and Guy's Hospital London, graduating in 1962. He undertook postgraduate clinical training and a fellowship in clinical immunology in London. In 1970 he was appointed Consultant Physician at Charing Cross Hospital, and since then he combined practice as a clinician in rheumatology and internal medicine with laboratory-based immunological research at the Kennedy Institute of Rheumatology. He was appointed Professor of Immunology at Charing Cross Medical School in 1979, and from 1990 to 2002 he was Professor of Rheumatology and Scientific Director/Head of the Kennedy Institute of Rheumatology, at Imperial College, London, until his retirement. His 'bench to bedside' research, in collaboration with Marc Feldmann, which commenced in 1985, has resulted in the development of anti-TNF immunotherapy of RA. Following the identification of TNF as a therapeutic target and translation of anti-TNF therapy to the clinic, Professors Maini and Feldmann have been jointly awarded many prizes, notably the Crafoord Prize by the Royal Swedish Academy of Sciences, the Lasker Prize for Clinical Research, the Dr Paul Janssen Award for Biomedical Research, the Ernst Schering Prize, and the Canada Gairdner International Award. He

has been Emeritus Professor of Rheumatology at Imperial College, London since 2002, and a Visiting Professor to the Kennedy Institute of Rheumatology at Oxford.

Professor Jeremy Saklatvala

BSc MBBS PhD FRCP FMedSci (b. 1943) qualified in medicine at University College Hospital London in 1968, specialized in Rheumatology at the MRC unit at the Canadian Red Cross Hospital at Taplow (UK) and in Glasgow at the Centre for Rheumatic Diseases. He obtained his PhD in Biochemistry on plasma protease inhibitors in 1975 at Strathclyde University, and was subsequently Arthritis Research Campaign Senior Research Fellow at Strangeways Research Laboratory in Cambridge working on the early characterization and purification of IL-1 and its role in cartilage and bone destruction. He also identified the similar actions of TNF and the common IL-1 receptor. He moved to the Babraham Research Institute in 1993 and focused on intracellular signalling mechanisms of IL-1 and TNF, particularly discovering the p38 MAPK pathway. He moved to the Kennedy Institute of Rheumatology in London in 1996, retiring as Deputy Director in 2014 following the move of the Kennedy Institute to be part of the University of Oxford in 2013.

Professor Josef Smolen

MD FRCP graduated from the Medical Faculty of University of Vienna, and trained at the Institute of Immunology, University of Vienna, under Carl Steffen. He undertook his residency in Internal Medicine at the 2nd Department of Medicine under Georg Geyer, and performed a research fellowship at the National Institutes of Health, National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases, Arthritis and Rheumatism Branch (headed by John Decker) in the Section for Cellular Immunology under Alfred D. Steinberg. He is now Professor of Internal Medicine and Chairman of the Division of Rheumatology and Department of Medicine 3 at the Medical University of Vienna. He is also Head of the 2nd Department of Medicine – Center of Rheumatic Diseases at Hietzing Hospital, Vienna. Professor Smolen has served as President of the EULAR (2003–2005), as President of the Austrian Society of Rheumatology (2005–2007), and as President of the Austrian Society of Immunology (2007–2009). Since 2003 he has been a Member of the Austrian Academy of Sciences and since 2007 of the German Academy of Sciences. He became a Fellow of the Royal Society of Physicians in 2008 and Honorary Doctor of the

University of Lund in 2010. He is Editorial Board Member of several professional journals and has edited textbooks and handbooks in the field of rheumatology.

Professor Tilli Tansey

OBE PhD PhD DSc HonMD HonFRCP FMedSci (b. 1953) graduated in zoology from the University of Sheffield in 1974, and obtained her PhD in *Octopus* neurochemistry in 1978. She worked as a neuroscientist in the Stazione Zoologica Naples, the Marine Laboratory in Plymouth, the MRC Brain Metabolism Unit, Edinburgh, and was a Multiple Sclerosis Society Research Fellow at St Thomas' Hospital, London (1983–1986). After a short sabbatical break at the Wellcome Institute for the History of Medicine (WIHM), she took a second PhD in medical history on the career of Sir Henry Dale, and became a member of the academic staff of the WIHM, later the Wellcome Trust Centre for the History of Medicine at UCL. She became Professor of the History of Modern Medical Sciences at UCL in 2007 and moved to QMUL, with the same title, in 2010. With the late Sir Christopher Booth she created the History of Twentieth Century Medicine Group in the early 1990s, now the History of Modern Biomedicine Research Group at QMUL.

Dr Marcos Vidal

PhD (1974–2016) graduated in biology from the National University of Rosario (UNR), Argentina, and obtained a PhD in vitamin D metabolism in macrophages at the UNR in 2003. He undertook his postdoctoral studies in the laboratories of Ross Cagan in Washington University in Saint Louis (2003–2007) and in Mount Sinai Medical School (2007–2009), where his research focused on oncogenic kinases in *Drosophila* models for cancer. In 2009 he started a group leader position at the Cancer Research UK Beatson Institute in Glasgow to study tumour-immune interactions in *Drosophila*. He was also elected a member of the Royal Society of Scotland Young Academy of Scotland. In recognition of his work and standing in the field, in July 2015, Dr Vidal was unanimously recommended for promotion to Senior Group Leader by a panel of external experts, and was also subsequently promoted to Professor at the University of Glasgow.

Professor Henning Walczak

MSc PhD (b. 1966) graduated in biology from the University of Bielefeld in 1992, and obtained a PhD from the same university in 1995 for work carried out at the German Cancer Research Centre (DKFZ, Heidelberg, Germany) on activation-induced T cell death. He worked at Immunex Corporation in Seattle (WA, USA) for two years (1996–1997) before returning to DKFZ in 1998, where he became Group Leader in 2000 following receipt of a BioFuture Prize awarded by the German Ministry for Science and Education. In 2007 he was appointed Chair of Tumour Immunology at Imperial College London, joining UCL's Cancer Institute in 2013 where he currently acts as Head of the Department & Chair of Cancer Biology and Scientific Director of the Cancer Research UK – UCL Centre. In 2012 he was awarded both an ERC Advanced Grant and a Wellcome Trust Senior Investigator Award. His research focuses on cell death and ubiquitin in inflammation, cancer, and (auto)immunity. He is particularly interested in unravelling the mechanisms of how the TNF, CD95, and TRAIL death receptor-ligand systems, but also other TNF superfamily receptor-ligand systems, are regulated and how they impact cancer cell survival, cancer-related inflammation, and cancer immunity.

Associate Professor Richard Williams

BSc MSc PhD (b. 1956) graduated in agriculture at the University of Wales, Bangor, in 1979, and obtained an MSc in animal parasitology in the same institution in 1981. He worked briefly with R. S. (Bill) Bray at Imperial College at Silwood Park, Ascot, on immunity to leishmaniasis (1984–1985) before joining the Babraham Institute to work with Tony Whyte on the immunotherapy of RA. In 1989 he moved to the Kennedy Institute of Rheumatology in London to work as a research assistant with David Williams before obtaining a PhD in immunology in 1995 at the University of London under the supervision of Tiny Maini. His work with animal models played an important role in the development of TNFα blockade as a therapeutic strategy for RA and since then he has continued to do translational research. In 2000 the Kennedy Institute of Rheumatology was incorporated into Imperial College London and Richard Williams was appointed as Senior Lecturer in 2007, and then promoted to Reader in 2010. The Kennedy Institute joined the University

of Oxford in 2011 and he was appointed as PI and Associate Professor in 2014.

Dr James (Jim) Woody

MD PhD is a General Partner of Latterell Venture Partners, a venture capital firm that invests in early and late stage biopharmaceutical, instrumentation, and medical device companies, since November 2005. Dr Woody brings more than 25 years of biomedical research and management experience to the partnership. He was formerly President of Roche Bioscience (former Syntex) in Palo Alto, California (1996–2004), where he had responsibility for bioscience research and clinical development. Previously, Dr Woody served as Chief Scientific Officer and Senior Vice President of R&D for Centocor, now Janssen Biotech (1991–1996). While at Centocor, Dr Woody, along with colleagues, developed 'Remicade', the first of the TNF inhibitor biologics, the first effective therapy for RA, Crohn's disease, and psoriasis. Prior to Centocor, Dr Woody served as a US Navy Medical Officer, was the cofounder with Navy colleagues of the National Marrow Donor Program. He retired as Commanding Officer and Director, US Naval Medical Research and Development Command in Bethesda, Maryland. Dr Woody currently serves as a member of the board of directors of four LVP companies, and was the founding CEO and Chairman of the Board of OncoMed Pharmaceuticals, Inc. (2004–2014) (IPO with NASDAQ: OMED 2013). Dr Woody is a member of the Board of Directors of the Lucille Packard (Stanford) Children's Hospital (LPCH) in Palo Alto, California. He holds an MD from Loma Linda University, trained in Pediatric Immunology at Duke University and Boston Children's Hospital (Harvard University), and earned a PhD in Immunology from the University of London, England. Dr Woody has authored or co-authored over 140 publications.

References*

Abraham E, Anzueto A, Gutierrez G, *et al*. (1998) Double-blind randomised controlled trial of monoclonal antibody to human tumour necrosis factor in treatment of septic shock. NORASEPT II Study Group. *Lancet* **351:** 929–933.

Alexander W R M. (1967–1968) The rheumatoid factor. *Res Medica* **6:** 17–19.

Balkwill F. (2009) Tumour necrosis factor and cancer. *Nature Reviews Cancer* **9:** 361–371.

Balkwill F R, Burke F. (1989) The cytokine network. *Immunology Today* **10:** 299–304.

Balkwill F R, Ward B G, Fiers W. (1987) Effects of tumour necrosis factor on human tumour xenografts in nude mice. *Ciba Foundation Symposium* **131:** 154–169.

Beeson P B. (1948) Temperature-elevating effect of a substance obtained from polymorphonuclear leucocytes. *Journal of Clinical Investigation* **27:** 524.

Beutler B, Cerami A. (1986) Cachectin and tumour necrosis factor as two sides of the same biological coin. *Nature* **320:** 584–588.

Beutler B, Milsark I W, Cerami A C. (1985) Passive immunization against cachectin/tumor necrosis factor protects mice from lethal effect of endotoxin. *Science* **229:** 869–871.

Bloom B R, Bennett B. (1966) Mechanism of a reaction *in vitro* associated with delayed-type hypersensitivity. *Science* **153:** 80–82.

Blumberg P M. (1988) Protein kinase C as the receptor for the phorbol ester tumor promoters: sixth Rhoads memorial award lecture. *Cancer Research* **48:** 1–8.

Bodmer W F. (1980) The HLA system and disease. The Oliver Sharpey Lecture 1979. *Journal of the Royal College of Physicians* **14:** 43–50.

* Please note that references with four or more authors are cited using the first three names followed by *'et al.'*. References with *'et al.'* are organized in chronological order, not by second author, so as to be easily identifiable from the footnotes.

Bogg A. (1958) Skin complications of gold treatment. *Acta Rheumatologica Scandinavica* **4:** 86–97.

Bonavida B, Gifford G E, Kirchner H, *et al*. (eds) (1988) Tumor necrosis factor/cachectin and related cytokines. In: *International Conference on Tumor Necrosis Factor and Related Cytotoxins.* Heidelberg, Germany, 14–18 September, 1987. Basel: Karger.

Bottazzo G F, Pujol-Borrell R, Hanafusa T, *et al*. (1983) Role of aberrant HLA-DR expression and antigen presentation in induction of endocrine autoimmunity. *Lancet* **322:** 1115–1119.

Bourke B E, Moss I K, Mumford P, *et al*. (1982) The complement fixing ability of putative circulating immune complexes in rheumatoid arthritis and its relationship to extra-articular disease. *Clinical and Experimental Immunology* **48:** 726–732.

Breedveld F C. (1998) Monoclonal antibodies to CD4. *Rheumatic Diseases Clinics of North America* **24:** 567–578.

Brennan F M, Chantry D, Jackson A, *et al*. (1989) Inhibitory effect of TNFα antibodies on synovial cell interleukin-1 production in rheumatoid arthritis. *Lancet* **334:** 244–247.

Buchan G, Barrett K, Fujita T, *et al*. (1988) Detection of activated T cell products in the rheumatoid joint using cDNA probes to interleukin-2 (IL-2), IL-2 receptor and IFN-γ. *Clinical and Experimental Immunology* **71:** 295–301.

Buchan G, Barrett K, Turner M, *et al*. (1988) Interleukin-1 and tumour necrosis factor mRNA expression in rheumatoid arthritis: prolonged production of IL-1 alpha. *Clinical and Experimental Immunology* **73:** 449–455.

Burnet F M. (1961) Immunological recognition of self. *Science* **133:** 307–311.

Carswell E A, Old L J, Kassel R L, *et al*. (1975) An endotoxin-induced serum factor that causes necrosis of tumors. *Proceedings of the National Academy of Sciences* **72:** 3666–3670.

Charles P, Elliott M J, Davis D, *et al*. (1999) Regulation of cytokines, cytokine inhibitors, and acute-phase proteins following anti-TNF-alpha therapy in rheumatoid arthritis. *Journal of Immunology* **163:** 1521–1528.

Christie D A, Tansey E M. (eds) (2007) *The Discovery, Use and Impact of Platinum Salts as Chemotherapy Agents for Cancer*. Wellcome Witnesses to Twentieth Century Medicine, vol. 30. London: Wellcome Trust Centre for the History of Medicine at UCL.

Cohen J, Carlet J; International Sepsis Trial Study Group. (1996) INTERSEPT: an international, multicenter, placebo-controlled trial of monoclonal antibody to human tumor necrosis factor-alpha in patients with sepsis. *Critical Care Medicine* **24:** 1431–1440.

Coley W B. (1893) The treatment of malignant tumors by repeated inoculations of erysipelas: with a report of ten original cases. *American Journal of Medical Sciences* **105:** 487–511.

Cordero J B, Macagno J P, Stefanatos R K, *et al*. (2010) Oncogenic Ras diverts a host TNF tumor suppressor activity into tumor promoter. *Developmental Cell* **18:** 999–1011.

Courtenay J S, Dallman M J, Dayan A D, *et al*. (1980) Immunisation against heterologous type II collagen induces arthritis in mice. *Nature* **283:** 666–668.

Creagan E T, Kovach J S, Moertel C G, *et al*. (1988) A phase I clinical trial of recombinant human tumor necrosis factor. *Cancer* **62:** 2467–2471.

Creasey A A, Doyle L V, Reynolds M T, *et al*. (1987) Biological effects of recombinant human tumor necrosis factor and its novel muteins on tumor and normal cell lines. *Cancer Research* **47:** 145–149.

Crusz S M, Balkwill F R. (2015) Inflammation and cancer: advances and new agents. *Nature Reviews Clinical Oncology* **12:** 584–596.

David J R. (1966) Delayed hypersensitivity *in vitro*: its mediation by cell-free substances formed by lymphoid cell-antigen interaction. *Proceedings of the National Academy of Sciences* **56:** 72–77.

Dayer J M. (2002) The saga of the discovery of IL-1 and TNF and their specific inhibitors in the pathogenesis and treatment of rheumatoid arthritis. *Joint, Bone, Spine* **69:** 123–132.

Dayer J M, Beutler B, Cerami A. (1985) Cachectin/tumor necrosis factor stimulates collagenase and prostaglandin E_2 production by human synovial cells and dermal fibroblasts. *Journal of Experimental Medicine* **162:** 2163–2168.

Di Giovine F S, Malawista S E, Nuki G, *et al*. (1987) Interleukin 1 (IL 1) as a mediator of crystal arthritis. Stimulation of T cell and synovial fibroblast mitogenesis by urate crystal-induced IL 1. *Journal of Immunology* **138:** 3213–3218.

Di Giovine F S, Nuki G, Duff G W. (1988) Tumour necrosis factor in synovial exudates. *Annals of the Rheumatic Diseases* **47:** 768–772.

Dinarello C A, Cannon J G, Wolff S M, *et al*. (1986) Tumor necrosis factor (cachectin) is an endogenous pyrogen and induces production of interleukin 1. *Journal of Experimental Medicine* **163:** 1433–1450.

Dingle J T. (1979) Heberden oration 1978. Recent studies on the control of joint damage: the contribution of the Strangeways Research Laboratory. *Annals of the Rheumatic Diseases* **38:** 201–214.

Dumonde D C, Wolstencroft R A, Panayi G S, *et al*. (1969) "Lymphokines": non-antibody mediators of cellular immunity generated by lymphocyte activation. *Nature* **224:** 38–42.

Eisenhauer E A, Therasse P, Bogaerts J, *et al*. (2009) New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *European Journal of Cancer* **45:** 228–247.

Elliott M J, Maini R N, Feldmann M, *et al*. (1993) Treatment of rheumatoid arthritis with chimeric monoclonal antibodies to tumor necrosis factor α. *Arthritis & Rheumatism* **36:** 1681–1690.

Elliott M J, Maini R N, Feldmann M, *et al*. (1994a) Randomised double-blind comparison of chimeric monoclonal antibody to tumour necrosis factor α (cA2) versus placebo in rheumatoid arthritis. *The Lancet* **344:** 1105–1110.

Elliott M J, Maini R N, Feldmann M, *et al*. (1994b) Repeated therapy with monoclonal antibody to tumour necrosis factor α (cA2) in patients with rheumatoid arthritis. *The Lancet* **344:** 1125–1127.

Emmrich F, Horneff G, Becker W, *et al*. (1991) An anti-CD4 antibody for treatment of chronic inflammatory arthritis. *Agents and Actions Supplements* **32:** 165–170.

Engelmann H, Aderka D, Rubinstein M, *et al*. (1989) A tumor necrosis factor-binding protein purified to homogeneity from human urine protects cells from tumor necrosis factor toxicity. *Journal of Biological Chemistry* **264:** 11974–11980.

Exley A R, Cohen J, Buurman W, *et al.* (1990) Monoclonal antibody to TNF in severe septic shock. *Lancet* **335:** 1275–1277.

Feldmann M. (2009) Translating molecular insights in autoimmunity into effective therapy. *Annual Review of Immunology* **27:** 1–27.

Feldmann M, Maini R N. (2003) Lasker Clinical Medical Research Award. TNF defined as a therapeutic target for rheumatoid arthritis and other autoimmune diseases. *Nature Medicine* **9:** 1245–1250.

Fell H B, Glauert A M, Barratt M E, *et al.* (1976) The pig synovium. I. The intact synovium *in vivo* and in organ culture. *Journal of Anatomy* **122:** 663–680.

Flick D A, Gifford G E. (1984) Comparison of *in vitro* cell cytotoxic assays for tumor necrosis factor. *Journal of Immunological Methods* **68:** 167–175.

Furst D E, Emery P. (2014) Rheumatoid arthritis pathophysiology: update on emerging cytokine and cytokine-associated cell targets. *Rheumatology* **53:** 1560–1569.

Genestier L, Paillot R, Fournel S, *et al.* (1998) Immunosuppressive properties of methotrexate: apoptosis and clonal deletion of activated peripheral T cells. *Journal of Clinical Investigation* **102:** 322–328.

Gerlach B, Cordier S M, Schmukle A C, *et al.* (2011) Linear ubiquitination prevents inflammation and regulates immune signalling. *Nature* **471:** 591–596.

Gough S C, Simmonds M J. (2007) The HLA region and autoimmune disease: associations and mechanisms of action. *Current Genomics* **8:** 453–465.

Gray P W, Barrett K, Chantry D, *et al.* (1990) Cloning of human tumor necrosis factor (TNF) receptor cDNA and expression of recombinant soluble TNF-binding protein. *Proceedings of the National Academy of Sciences* **87:** 7380–7384.

Grewal I S. (2009) Overview of TNF superfamily: a chest full of potential therapeutic targets. *Advances in Experimental Medicine and Biology* **647:** 1–7.

Harrison M L, Obermueller E, Maisey N R, *et al.* (2007) Tumor necrosis factor α as a new target for renal cell carcinoma: two sequential phase II trials of infliximab at standard and high dose. *Journal of Clinical Oncology* **25:** 4542–4549.

Hench P S, Kendall E C, Slocumb C H, *et al*. (1949) The effect of a hormone of the adrenal cortex (17-hydroxy-11-dehydrocorticosterone: compound E) and of pituitary adrenocortical hormone in arthritis: preliminary report. *Annals of the Rheumatic Diseases* **8:** 97–104.

Horneff G, Burmester G R, Emmrich F, *et al*. (1991) Treatment of rheumatoid arthritis with an anti-CD4 monoclonal antibody. *Arthritis & Rheumatism* **34:** 129–140.

Jacob C O, McDevitt H O. (1988) Tumour necrosis factor-α in murine autoimmune 'lupus' nephritis. *Nature* **331:** 356–358.

Kalliolias G D, Ivashkiv L B. (2016) TNF biology, pathogenic mechanisms and emerging therapeutic strategies. *Nature Reviews Rheumatology* **12:** 49–62.

Kempeni J. (1999) Preliminary results of early clinical trials with the fully human anti-TNFα monoclonal antibody D2E7. *Annals of the Rheumatic Diseases* **58s:** I70–I72.

Klareskog L, Holmdahl R, Larsson E, *et al*. (1983) Role of T lymphocytes in collagen II induced arthritis in rats. *Clinical and Experimental Immunology* **51:** 117–125.

Köhler G, Milstein C. (1975) Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* **256:** 495–497.

Lawrence J S. (1953) Factors in gold dosage and toxicity in rheumatoid arthritis. *Annals of the Rheumatic Diseases* **12:** 129–135.

Liedtke C, Plümpe J, Kubicka S, *et al*. (2002) Jun kinase modulates tumor necrosis factor-dependent apoptosis in liver cells. *Hepatology* **36:** 315–325.

Londei M, Bottazzo G F, Feldmann M. (1985) Human T-cell clones from autoimmune thyroid glands: specific recognition of autologous thyroid cells. *Science* **228:** 85–89.

Londei M, Lamb J R, Bottazzo G F, *et al*. (1984) Epithelial cells expressing aberrant MHC class II determinants can present antigen to cloned human T cells. *Nature* **312:** 639–641.

Londei M, Savill C M, Verhoef A, *et al*. (1989) Persistence of collagen type II-specific T-cell clones in the synovial membrane of a patient with rheumatoid arthritis. *Proceedings of the National Academy of Sciences* **86:** 636–640.

Lorenz H M. (2002) Technology evaluations: adalimumab, Abbott laboratories. *Current Opinion in Molecular Therapeutics* **4:** 185–190.

Luqmani R A, Palmer R G, Bacon P A. (1990) Azathioprine, cyclophosphamide and chlorambucil. *Baillière's Clinical Rheumatology* **4:** 595–619.

Maini R N, Breedveld F C, Kalden J R, *et al*. (1998) Therapeutic efficacy of multiple intravenous infusions of anti-tumor necrosis factor α monoclonal antibody combined with low-dose weekly methotrexate in rheumatoid arthritis. *Arthritis & Rheumatism* **41:** 1552–1563.

Maini R N, Bryceson A D, Wolstencroft R A, *et al*. (1969) Lymphocyte mitogenic factor in man. *Nature* **224:** 43–44.

Maini R N, Elliott M J, Brennan F M, *et al*. (1995) Monoclonal anti-TNF alpha antibody as a probe of pathogenesis and therapy of rheumatoid disease. *Immunological Reviews* **144:** 195–223.

Malik S T, Griffin D B, Fiers W, *et al*. (1989) Paradoxical effects of tumour necrosis factor in experimental ovarian cancer. *International Journal of Cancer* **44:** 918–925.

Marmenout A, Fransen L, Tavernier J, *et al*. (1985) Molecular cloning and expression of human tumor necrosis factor and comparison with mouse tumor necrosis factor. *European Journal of Biochemistry* **152:** 515–522.

Miles D W, Happerfield L C, Naylor M S, *et al*. (1994) Expression of tumour necrosis factor (TNF alpha) and its receptors in benign and malignant breast tissue. *International Journal of Cancer* **56:** 777–782.

Mitchison N A. (1964) Induction of immunological paralysis in two zones of dosage. *Proceedings of the Royal Society of London, Series B* **161:** 275–292.

Mohler KM, Torrance DS, Smith CA, *et al*. (1993) Soluble tumor necrosis factor (TNF) receptors are effective therapeutic agents in lethal endotoxemia and function simultaneously as both TNF carriers and TNF antagonists. *Journal of Immunology* **151:** 1548–1561.

Moore R J, Owens D M, Stamp G, *et al*. (1999) Mice deficient in tumor necrosis factor-α are resistant to skin carcinogenesis. *Nature Medicine* **5:** 828–831.

National Toxicology Program. (1996) NTP comparative initiation/promotion skin paint studies of B6C3F$_1$ mice, Swiss (CD-1®) mice, and SENCAR mice. *National Toxicology Program Technical Reports Series* **441:** 1–201.

Naylor M S, Stamp G W, Foulkes W D, *et al*. (1993) Tumor necrosis factor and its receptors in human ovarian cancer. Potential role in disease progression. *Journal of Clinical Investigation* **91:** 2194–2206.

Old L J. (1988) Tumor necrosis factor. *Scientific American* **258:** 59–60, 69–75.

Panacek E A, Marshall J C, Albertson T E, *et al*. (2004) Efficacy and safety of the monoclonal anti-tumor necrosis factor antibody F(ab')2 fragment afelimomab in patients with severe sepsis and elevated interleukin-6 levels. *Critical Care Medicine* **32:** 2173–2182.

Pasparakis M, Alexopoulou L, Episkopou V, *et al*. (1996) Immune and inflammatory responses in TNF alpha-deficient mice: a critical requirement for TNFα in the formation of primary B cell follicles, follicular dendritic cell networks and germinal centers, and in the maturation of the humoral immune response. *Journal of Experimental Medicine* **184:** 1397–1411.

Pestka S. (2007) The interferons: 50 years after their discovery, there is much more to learn. *Journal of Biological Chemistry* **282:** 20047–20051.

Prentice H G, Blacklock H A, Janossy G, *et al*. (1982) Use of anti-T-cell monoclonal antibody OKT3 to prevent acute graft-versus-host disease in allogeneic bone-marrow transplantation for acute leukaemia. *Lancet* **319:** 700–703.

Pullar T, Hunter J A, Capell H A. (1987) Effect of sulphasalazine on the radiological progression of rheumatoid arthritis. *Annals of the Rheumatic Diseases* **46:** 398–402.

Rankin E C, Choy E H, Kassimos D, *et al*. (1995) The therapeutic effects of an engineered human anti-tumour necrosis factor alpha antibody (CDP571) in rheumatoid arthritis. *British Journal of Rheumatology* **34:** 334–342.

Rau R, Sander O, van Riel P, *et al*. (2003) Intravenous human recombinant tumor necrosis factor receptor p55-Fc IgG1 fusion protein Ro 45-2081 (lenercept): a double blind, placebo controlled dose-finding study in rheumatoid arthritis. *Journal of Rheumatology* **30:** 680–690.

Reinhart K, Wiegand-Löhnert C, Grimminger F, *et al*. (1996) Assessment of the safety and efficacy of the monoclonal anti-tumor necrosis factor antibody-fragment, MAK 195F, in patients with sepsis and septic shock: a multicenter, randomized, placebo-controlled, dose-ranging study. *Critical Care Medicine* **24:** 733–742.

Research Sub-Committee of the Empire Rheumatism Council. (1957) Empire Rheumatism Council: multi-centre controlled trial comparing cortisone acetate and acetyl salicylic acid in the long-term treatment of rheumatoid arthritis; results of three years' treatment. *Annals of the Rheumatic Diseases* **16:** 277–289.

Research Sub-Committee of the Empire Rheumatism Council. (1961) Gold therapy in rheumatoid arthritis: final report of a multicentre controlled trial. *Annals of the Rheumatic Diseases* **20:** 315–334.

Rice T W, Wheeler A P, Morris P E, *et al*. (2006) Safety and efficacy of affinity-purified, anti-tumor necrosis factor-alpha, ovine Fab for injection (CytoFab) in severe sepsis. *Critical Care Medicine* **34:** 2271–2281.

Rickard J A, Anderton H, Etemadi N, *et al*. (2014) TNFR1-dependent cell death drives inflammation in Sharpin-deficient mice. *eLife* **3:** e03464.

Rose H M, Ragan C, Pearce E, *et al*. (1948) Differential agglutination of normal and sensitized sheep erythrocytes by sera of patients with rheumatoid arthritis. *Proceedings of the Society of Experimental Biology and Medicine* **68:** 1–6.

Sarvetnick N, Liggitt D, Pitts S L, *et al*. (1988) Insulin-dependent diabetes mellitus induced in transgenic mice by ectopic expression of class II MHC and interferon-gamma. *Cell* **52:** 773–782.

Selby P, Hobbs S, Viner C, *et al*. (1987) Tumour necrosis factor in man: clinical and biological observations. *British Journal of Cancer* **56:** 803–808.

Shealy D J, Visvanathan S. (2008) Anti-TNF antibodies: lessons from the past, roadmap for the future. *Handbook of Experimental Pharmacology* **181:** 101–129.

Silverstein A M. (2001). Autoimmunity *versus* horror autotoxicus: the struggle for recognition. *Nature Immunology* **2:** 279–281.

Smolen J S, Aletaha D, Keystone E. (2005) Superior efficacy of combination therapy for rheumatoid arthritis: fact or fiction? *Arthritis & Rheumatism* **52:** 2975–2983.

Smolen J S, Landewé R, Breedveld F C, *et al.* (2014) EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2013 update. *Annals of the Rheumatic Diseases* **73:** 492–509.

Taniguchi T, Fujii-Kuriyama Y, Muramatsu M. (1980) Molecular cloning of human interferon cDNA. *Proceedings of the National Academy of Sciences* **77:** 4003–4006.

Tansey EM, Catterall P P, Christie D A, et al. (eds) (1997) Self and Non-Self: a History of Autoimmunity. *Wellcome Witnesses to Twentieth Century Medicine,* vol. 1. London: The Wellcome Trust, 35-66, available online at www.histmodbiomed.org/witsem/vol1 (accessed 13 December 2016).

Taylor P C, Peters A M, Paleolog E, *et al.* (2000) Reduction of chemokine levels and leukocyte traffic to joints by tumor necrosis factor α blockade in patients with rheumatoid arthritis. *Arthritis & Rheumatism* **43:** 38–47.

The Lenercept Multiple Sclerosis Study Group and The University of British Columbia MS/MRI Analysis Group. (1999) TNF neutralization in MS: results of a randomized, placebo-controlled multicenter study. *Neurology* **53:** 457–465.

van der Lubbe P A, Reiter C, Breedveld F C, *et al.* (1993) Chimeric CD4 monoclonal antibody cM-T412 as a therapeutic approach to rheumatoid arthritis. *Arthritis & Rheumatism* **36:** 1375–1379.

van Oosten B W, Lai M, Hodgkinson S, *et al.* (1997) Treatment of multiple sclerosis with the monoclonal anti-CD4 antibody cM-T412: results of a randomized, double-blind, placebo-controlled, MR-monitored phase II trial. *Neurology* **49:** 351–357.

von Karstedt S, Conti A, Nobis M, *et al.* (2015) Cancer cell-autonomous TRAIL-R signaling promotes KRAS-driven cancer progression, invasion, and metastasis. *Cancer Cell* **27:** 561–573.

Waalen K, Duff G W, Førre O, *et al.* (1986) Interleukin 1 activity produced by human rheumatoid and normal dendritic cells. *Scandinavian Journal of Immunology* **23:** 365–371.

Waaler E. (1940) On the occurrence of a factor in human serum activating the specific agglutination of sheep blood corpuscles. *Acta Pathologica et Microbiologica Scandinavica* **17:** 172–188.

Ward J R. (1985) Historical perspective on the use of methotrexate for the treatment of rheumatoid arthritis. *Journal of Rheumatology* **12s:** 3–6.

Wick W, Fricke H, Junge K, *et al*. (2014) A phase II, randomized, study of weekly APG101+reirradiation versus reirradiation in progressive glioblastoma. *Clinical Cancer Research* **20:** 6304–6313.

Williams R O, Whyte A. (1989) A comparison of anti-type II collagen antibody titres and isotype profiles in pregnant and virgin DBA/1 mice with collagen-induced arthritis. *Journal of Reproductive Immunology* **15:** 229–239.

Williams R O, Whyte A, Waldmann H. (1989) Resistance to collagen-induced arthritis in DBA/1 mice by intraperitoneal administration of soluble type II collagen involves both CD4+ and CD8+ T lymphocytes. *Autoimmunity* **4:** 237–245.

Williams R O, Mason L J, Feldmann M, *et al*. (1994) Synergy between anti-CD4 and anti-tumor necrosis factor in the amelioration of established collagen-induced arthritis. *Proceedings of the National Academy of Sciences* **91:** 2762–2766.

Williams R O, Mauri C, Mason L J, *et al*. (1998) Therapeutic actions of cyclosporine and anti-tumor necrosis factor alpha in collagen-induced arthritis and the effect of combination therapy. *Arthritis & Rheumatism* **41:** 1806–1812.

Willkens R F. (1985) Short term efficacy of methotrexate in the treatment of rheumatoid arthritis. *Journal of Rheumatology* **12s:** 21–24.

Wolff S M, Auron P E, Dinarello C A, *et al*. (1986) Clone controversy at Immunex. *Nature* **319:** 270.

Wright P. (2013) Deborah Doniach. *Lancet* **363:** 995.

Zhang G, Li J, Purkayastha S, *et al*. (2013) Hypothalamic programming of systemic ageing involving IKK-β, NF-κB and GnRH. *Nature* **497:** 211–216.

Ziff M. (1965) Heberden Oration, 1964: Some immunologic aspects of the connective tissue diseases. *Annals of the Rheumatic Diseases* **24:** 103–115.

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OTHER PUBLICATIONS

Technology transfer in Britain: The case of monoclonal antibodies
Tansey E M, Catterall P P. (1993) *Contemporary Record* **9**: 409–44.

Monoclonal antibodies: A witness seminar on contemporary medical history
Tansey E M, Catterall P P. (1994) *Medical History* **38**: 322–7.

Chronic pulmonary disease in South Wales coalmines: An eye-witness account of the MRC surveys (1937–42)
P D'Arcy Hart, edited and annotated by E M Tansey. (1998)
Social History of Medicine **11**: 459–68.

Ashes to Ashes – The history of smoking and health
Lock S P, Reynolds L A, Tansey E M. (eds) (1998) Amsterdam: Rodopi BV, 228pp. ISBN 90420 0396 0 (Hfl 125) (hardback). Reprinted 2003.

Witnessing medical history. An interview with Dr Rosemary Biggs
Professor Christine Lee and Dr Charles Rizza (interviewers). (1998)
Haemophilia **4**: 769–77.

Witnessing the Witnesses: Pitfalls and potentials of the Witness Seminar in twentieth century medicine
Tansey E M, in Doel R, Søderqvist T. (eds) (2006) *Writing Recent Science: The historiography of contemporary science, technology and medicine*. London: Routledge: 260–78.

The Witness Seminar technique in modern medical history
Tansey E M, in Cook H J, Bhattacharya S, Hardy A. (eds) (2008) *History of the Social Determinants of Health: Global Histories, Contemporary Debates*. London: Orient Longman: 279–95.

Today's medicine, tomorrow's medical history
Tansey E M, in Natvig J B, Swärd E T, Hem E. (eds) (2009) *Historier om helse* (*Histories about Health,* in Norwegian). Oslo: *Journal of the Norwegian Medical Association:* 166–73.

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