European Science Foundation

Standing Committee for the European Medical Research Councils (EMRC)

ESF/EMRC EXPLORATORY WORKSHOP ON:

Chronic inflammation: basic mechanisms and therapeutic targets

London, United Kingdom

19-21 September 2001

Convened by: Y. Chernajovsky

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1. Executive summary

We recently held at The Novartis Foundation in London the ESF Exploratory workshop on:" Chronic inflammation: basic mechanisms and therapeutic targets".

Due to recent events in New York and general apprehension there were a number of last moment cancellations including: Prof. Paul Robbins (USA), Dr. Olivier Danos (France), Dr. Osvaldo Podhajcer (Argentina) Dr. Daniela Novick (Israel), Prof. George Kollias (Greece) and Prof. Hartmut Wekerle (Germany).
Prof. Bordignon (Italy) had a last moment operation but succeeded to send his colleague Dr. Sarah Marktel (training in London) as substitute. I nominated Prof. Francis Balkwill (London) to replace Dr. Podhajcer.

Understanding the budgetary constraints of this meeting but knowing the importance of the topics discussed and the possibility of establishing new links and collaborations, I nominated Chairpersons that did not formally present but participated in the Discussion Sessions and helped run each session on time. They were: Profs. Ian Sanderson, Bruce Kidd, Paul Winyard, Fionula Brennan, John Priestley, Brian Foxwell, Francis Balkwill and Dr. Rizgar Mageed. Also I took the educational and scientific opportunity to expose students and members of my Department to the speakers presentations. Members of my Department did not participate in any other activity as it was not budgeted to be so.

Originally, I planned that the discussion sessions will be divided according to disease interest groups where people from the field of gene therapy and molecular biology and cell signaling will move around the different interest groups. However, we held instead general discussion sessions from which several links and collaborations were set up (see below). All participants found the long Discussion Sessions (2-3 hours) was very fruitful and important.

It was decided that the Workshop was very useful and that should be held in two years time in France. Prof. Chatenoud and myself will like to co-organise it.

The following new potential collaborations were established:
1. Mageed, Wallach, Bujard and Follenzi
2. t'Hart, Toivanen, Holmdahl and Dayer
3. Holmdahl, Baker, Jorgensen and Tak
4. Priestley, Baker, Chernajovsky
5. Jorgensen and Chernajovsky

2. Scientific content of the event.

The areas of biomedical research covered included genetics, arthritis, multiple sclerosis, irritable bowel syndrome, diabetes, cancer and gene therapy. This workshop brought together a broad range of expertise including molecular biologists, geneticists, physicians, immunologists and neuroscientists.

The importance of genetic mapping and sequence of the genome was discussed by Weissenbach . He stressed that there are several regions of the human genome still lacking complete information and compared the approaches taken by the private sector and the publicly funded mapping projects. The importance of the genetic make up regarding the susceptibility to infection was addressed by Toivanen who showed that people with different genotypes contain different intestinal flora indicating a delicate balance between microorganisms and the host. He provided evidence that some of the cell wall components of some of these organisms could be involved in arthritis development as they can induce in vitro the production of TNF and MCP1, can be found in plasma and also could elicit arthritis in rats after injection. Holmdahl also focussed on the genetic contribution of genes in different phases of disease progression. By breeding congenic rat strains he maps the contribution of different genetic loci to the development of pristane-induced arthritis. Some genes appear to contribute to the acute phase or the chronic phase while other genes may contribute to both. The relevance of animal models of human disease was addressed also by other speakers. The importance of using outbred animals was stressed repeatedly as inbred strains represent genetically just one individual and conclusions should not be generalised. Indeed, in experimental allergic encephalomyelitis (EAE), the pathogenic antigenic peptide (MOG 14-36) utilises an MHC class II which is the same even in outbred marmosets (t'Hart) indicating that despite different genetic backgrounds, certain genes may have a dominant contribution to disease susceptibility to a defined pathogenic trigger. Not knowing the aetiology of most of human diseases to draw direct genetic correlations from particular genotypes appears to be premature but, it was stressed that it was an important issue.

Further differences, between mice and man, were discussed by Pellegrini, who described the response of naïve T cells to IFN type I and their differentiation to Th1 cells. Mice lack the STAT4 gene necessary for IFN-driven Th1 differentiation. Interestingly only naïve T cells respond but differentiated Th1 or Th2 cells do not. The molecular mechanism(s) controlling this lack of IFN effect on differentiated T cells does not appear to lie with the known signalling pathways. Yet, IFN responsive genes fail to become activated. This information is important as relates to the therapeutic actions of IFNs *in vivo*. Indicating that we do not really know what cells are the targets of the immunomodulatory therapeutic effects, possibly they are not differentiated T cells.

The importance of targeting T cells for therapeutic intervention in diabetes, was discussed by Chatenoud who indicated that using MEL14+ T cells transduced with the IL-4 gene potentiates their therapeutic capabilities in the NOD model. Powrie described the regulatory role of CD4+CD25+ CD45RB low T cells in a model of irritable bowel syndrome. These cells inhibit transfer of disease and their effect requires IL-10, TGFβ and CTLA-4. The presence of these regulatory T cells in other immune conditions is pursued by many groups. Whether these cells are antigen- specific or class II restricted is yet unknown.

Cytokines, as therapeutic targets, were discussed by several participants. The possible beneficial effects of IFNβ in rheumatoid arthritis (RA) was discussed by Tak, Dayer and Chernajovsky. The important role of IL-1 in cartilage degradation in arthritis as well as the role of other cytokines (TNF, IL-17, IL-18, OPG-L) in the pathogenic cascade was discussed by van den Berg. A clear predominant anti-inflammatory effect of anti-TNF whilst a chondroprotective effect of IL-1ra in RA was proposed and clinical combination therapy studies are now being put forward.

The potential therapeutic action of cytokines were shown to be dependent on the method of delivery as well as the target cell which expressed the gene product when delivered by gene therapy. Baker showed that IL-10 had therapeutic effects in EAE when delivered by engineered fibroblasts implanted intracraneally but no effect when delivered by adenovirus vectors. Chernajovsky addressed the possibility of delivering cytokines only to sites of inflammation using latent-fusion proteins in order to avoid pleiotropic effects on healthy tissues.

The pleiotropic actions of cytokines were exemplified by Balkwill who addressed the role of TNF as a facilitator of tumour growth in ovarian and skin cancer . Tumour stromal cells produce TNF, chemokines, angiogenic factors and metalloproteinases that facilitate tumour growth and migration. Targeting TNF with antibodies during the developing phase of skin cancer has therapeutic effects which may be mediated in part by inhibition of TNF-mediated MMP9 expression. Balkwill also touched on the role of chemokine receptor expression on cancer cells during the process of cancer metastasis. Wallach discussed the role of the TNF receptor family in controlling both innate and adaptive immunity. The important roles of the 'signalosome' and 'proteasome'in cell survival and death was described. The interactions of the TNF receptor complex with the signalosome via IKKγ (NEMO) was addressed. TNF- induced death requires the role of caspase 8 and FLIP regulates its activity. Data was presented from biochemical studies and gene knock-out mice.

T cells were shown to be important gene carrier devices both in cancer (Eshhar), diabetes (Chatenoud) and arthritis (Chernajovsky) and they could be retargeted to recognise either tumour antigens or cartilage components using chimeric receptors based on extracellularly expressed immunoglobulin scFv fragments and intracellular signalling domains. Marktel described an interesting method of controlling graft vs. host disease during allogeneic bone marrow transplantation to cancer patients using retrovirus expressing the suicidal HSV-tk /ganciclovir system. The immune response against HSV-tk gene expressing cells limits the long-term use of this system and alternative approaches are being investigated.

Cell-cell interactions are very important for the pathogenesis of disease. Dayer, showed that membrane preparations from activated T cells can induce secretion of IL-1 from monocytes/macrophages. Membranes from Th1 or Th2 activated T cells have different effects on cytokine secretion by monocytes. The former induces IL-1 and TNF whilst the latter induces IL1-ra. In search for factors regulating this cell-cell interaction they have isolated a human serum component that blocks this interaction, namely APO-1. The mechanism of inhibition is unknown but it appears to bind to the T cell membrane and not to the monocytes. Interestingly, APO-1 expression by the liver is reduced during inflammation processes.

Tissue repair of damaged cartilage and bone during arthritic disease was addressed by Jorgensen. The exploitation of mesenchymal stem cells that could differentiate into chondrocytes and osteoblasts was described. The length of growth factor expression *in vivo* was shown to be crucial to achieve optimal differentiation .

Gene transfer and expression systems harnessing properties of pathological events (hypoxia) were discussed by Kingsman and the use of the synthetic tetracycline-regulated systems for *ex vivo, in vivo* and in transgenic animals were described by Bujard.

Results from clinical trials in glioma and the potential of using replicating retrovirus as compared to adenovirus for their treatment was discussed by Klatzman . The recently developed lentiviral (HIV) vectors were discussed by Follenzi. Also Follenzi described the development of HIV vectors for liver-specific gene expression using the woodchuck hepatitis PRE element.

3. Final Programme

Wednesday 19 September 2001

08h00	*Breakfast (30 minutes)*
	Chairman: Brian Foxwell
08h30	**Welcome** *Yuti Chernajovsky, Convenor*
08h45	**Human genome project: past, present and future scientific and social issues** *Jean Weissenbach, France*
09h30	**TNF-mediated growth and death** *David Wallach, Israel*
10h15	**IFN-mediated signalling and gene regulation** *Sandra Pellegrini, France*
	Chairwoman: Fionula Brennan
11h00	*Coffee break (30 minutes)*
11h30	**Cytokine manipulation in arthritis models** *Wim van den Berg, The Netherlands*
12h15	*Lunch break (1 hour)*
13h15	**Primate models of auto-immune disease** *Bert t'Hart, The Netherlands*
14h00	**Use of cytokines and cytokine inhibitors in clinical trials of rheumatoid arthritis** *Paul P. Tak, The Netherlands*
	Chairman: Paul Winyard
14h45	**Exploiting the Hypoxia response in gene therapy and gene discovery** *Sue Kingsman, United Kingdom*
15h00	**Tetracycline-regulated vectors, development and uses** *Herman Bujard, Germany*
15h45	*Coffee break (30 minutes)*
	WORKING GROUP MEETINGS FOR COLLABORATIVE RESEARCH
18h00	*Meeting adjourns*

Thursday 20 September 2001

08h00	*Breakfast (30 minutes)*
	Chairman: John Priestley
08h30	**ESF/EMRC Presentation** *Yuti Chernajovsky on behalf of* *M. Minkowski, ESF Senior Scientific Secretary*
09h00	**Gene transfer for the treatment of experimental auto-immune encephalomyelitis** *David Baker, United Kingdom*
09h40	**Gene transfer in human gliomas, use of replicating retroviral vectors** *David Klatzmann, France*
	Chairman: Bruce Kidd
10h20	**Use of mesenchymal stem cells for tissue joint repair** *Christian Jorgensen, France*
11h00	*Coffee break (30 minutes)*
11h30	**Cell-cell interactions in inflammation** *Jean-Michel Dayer, Switzerland*
12h10	**Engineering molecules and cells for targeting sites of inflammation** *Yuti Chernajovsky, United Kingdom*
	Chairman: Ian Sanderson
12h50	**Regulatory T cells in the gut** *Fiona Powrie, United Kingdom*
13h30	*Lunch break (1 hour)*
14h10	**Controlling diabetes mellitus by immune and gene transfer approaches** *Lucienne Chatenoud, France*
14h50	**Genetic control of chronic inflammation** *Rikard Holmdahl, Sweden*
15h30	**Role of intestinal bacteria and their degradation products in the pathogenesis of rheumatoid arthritis** *Paavo Toivanen, Finland*
16h10	*Coffee break (30 minutes)*
	WORKING GROUP MEETINGS FOR COLLABORATIVE RESEARCH
18h00	*Meeting adjourns*

Friday 21 September 2001

08h00	*Breakfast (30 minutes)*
	Chairman: Rizgar Mageed
08h30	**Development and use of lentiviral vectors** *Antonia Follenzi, Italy*
	Chairwoman: Fran Balkwill
09h30	**Gene transfer to bone marrow cells: applications and limitations** *Sarah Marktel, Italy (on behalf of Claudio Bordignon)*
10h30	*Coffee break (30 minutes)*
11h00	**Development of T bodies for cancer therapy** *Zelig Eshhar, Israel*
12h00	**The links between inflammation and cancer** *Fran Balkwill, UK*
	WORKING GROUP MEETINGS FOR COLLABORATIVE RESEARCH
13h00	*Lunch / Meeting closes*

3. Assessment of the results, contribution to the future direction of the field

The meeting was a success. All participants actively contributed to the discussion sessions and several issues were brought up that need further investigations.

a. From the pathology/aetiology point of view it was agreed that information should be collated that links genetic susceptibility to pathogenic organisms and severity of diseases. Including viruses and bacteria. The importance of the gut flora as pathogen or as a potential regulator of the immune response needs following up in several diseases.

b. Also it was agreed that response or lack of response to therapy with drugs or with biologicals should be annotated in relation to genotypes. This latter approach was compared to finding the antibiotic resistant genes in bacteria. Such an approach will help elucidate pathways involved in the mechanism of action of drugs and predict populations of responders versus non-responders allowing for careful selection of patients for particular treatments. Routes of administration of biologicals should be carefully compared, as their primary and secondary biological effects may depend on which organs or lymphoid tissues they encounter first.

c. Due to the complexity of the cytokine network, the time dependent and cell / tissue / extracellular matrix dependent responses to particular cytokines, both in a normal immune response and under experimental therapies, it was felt that a microanatomical analysis supported by the development of bioinformatics tools will be necessary to understand pathogenic processes. Cellular composition analysis via microanatomical studies across time should be done both at the protein level by immunohistochemistry as well as by riboprobes and complemented by DNA/ RNA array studies. Animal models for this type of study are essential, as is the use of clinical material.

d. We lack a good definition at the molecular level of the reasons for chronic inflammation. We need a deeper understanding of the mechanisms leading to the resolution of inflammation to understand why in chronic inflammation these mechanisms fail. Is epitope spreading a reason for chronicity? Can early markers be characterized to develop diagnostic methods that will improve treatment? Do genotype changes reported in inflammation affect cell metabolism in such way that normal resolution of inflammation is compromised?

e. There is a need to develop additional avenues for therapy. The potential of using gene transfer and genetic engineering methods are showing promise in animal models. The careful analysis of possible side effects of long term biological therapy has to be undertaken. The majority of the animal models used are short term i.e. weeks to months whilst some chronic diseases may need treatment for many years. Further development of gene transfer methods that enable tissue specific or temporal regulated expression are needed.

4. Statistical information on participants

The age of the participants ranged from 34 to 63 and an average of 45.
There were 20 males and 8 females.
Countries of origin: UK (12), France (5), Israel (2), Switzerland (1), Finland (1), Sweden (1), The Netherlands (3), Germany (1), Italy (2).

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