Keeping hopes high

Workshop on the Molecular and Genetic Basis of Autoimmune Diseases: SLE and RA

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Introduction

This workshop gathered together 20 speakers and 26 participants to discuss recent progress in the understanding and management of systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA). SLE and RA are both remitting and relapsing systemic autoimmune diseases (AIDs), which are characterized by the production of pathological auto-antibodies that participate in chronic inflammation and tissue destruction. Other AIDs include type 1 diabetes and multiple sclerosis (MS) and, like all these diseases,

Unusually, for a meeting held under the auspices of the Instituto Juan March de Estudios e Investigaciones (Madrid, Spain), this workshop took place at the Fundação Calouste Gulbenkian in Lisbon, Portugal. It was held between 7 and 9 April 2003, and was organized by A. Coutinho, W. Haas and C. Martínez-A. The meeting was co-sponsored by the Instituto Gulbenkian de Ciência and EMBO.

the prevalence of SLE and RA continues to increase in western countries. However, neither has a cure been found nor is their aetiology well understood. The challenge of this workshop was to bring together clinicians, geneticists and fundamental immunologists for a discussion-orientated meeting. The subsequent reciprocal interactions provided a rare opportunity to integrate data from clinical and therapeutic perspectives with the molecular and cellular processes that form the basis of these diseases.

First born, first line: innate immunity

The defective clearance of apoptotic cells has been shown in a number of human SLE-like diseases that are associated with a mutation in the complement *C1q* gene. Whether similar defects are a general hallmark of SLE is often discussed, notably because this disease is characterized by the production of abnormally high titres of antibodies that bind to DNA and other nuclear molecules. K. Elkon (Seattle, WA, USA) reviewed recent progress in understanding the mechanisms that control the induction of apoptosis and the processing of dying cells (Kim *et al.*, 2003). Intriguingly, natural IgM antibodies seem to participate in the clearance of apoptotic cells, and it is therefore conceivable that patients with a distorted serum Ig repertoire (see below) could present secondary defects in this pathway.

In his presentation, J. Tschopp (Lausanne, Switzerland) introduced the concept of 'inflammasomes'. These are protein complexes whose formation is necessary to activate the pro-inflammatory caspases, and which operate downstream of the Toll-like receptors (TLRs) that are activated in response to microbial infection. The formation of inflammasomes is mediated by the newly identified NALPs (for NACHT, LRR and PYD domains), a subgroup of the intracellular protein family CATERPILLER (Tschopp *et al.*, 2003). The *NALP3* gene is highly expressed in peripheral blood lymphocytes and seems to be mutated within the NACHT domain in patients with various inflammatory diseases. This domain normally ensures auto-inhibition of the protein by promoting self-folding, and it is expected that patients carrying such mutations suffer from spontaneous inflammasome formation.

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Those seen may not be the sinner

Both SLE and RA pathologies are, at least in part, antibody-mediated because of the production of auto-antibodies that are reactive against self-antigens. A discussion was initiated to delineate the structural differences between pathological auto-antibodies and the natural, harmless auto-antibodies that are produced by normal individuals. As summarized by A. Coutinho (Oeiras, Portugal) the emerging conclusion was that pathological antibody production is antigen-driven and T-cell-dependent, and that they are secreted by large clones of cells. By contrast, natural antibodies are produced by small clones of B cells. To complete this statement, M. Weigert (Princeton, NJ, USA) reminded us that analyses of anti-nuclear antibodies (ANAs), a hallmark of SLE, revealed that the number of antibody specificities found at high titre and frequency in patient sera is limited, which implies stringent selection events.

Weigert also discussed a strikingly conserved feature of ANAs: like other DNA-binding proteins such as transcriptional regulators, antibodies that bind directly to DNA all contain at least one arginine residue at the recognition site. He further showed that a single Arg in the recognition domain of an antibody molecule is sufficient for it to bind to DNA. To fully appreciate the significance of these findings, one should keep in mind that new lymphocytes are continuously being produced throughout adult life, all of which express a different antigen receptor. The mechanism that allows the production of such diverse molecules has an element of randomness; it is a combinatorial rearrangement of several gene segments, ligated to each other with an imprecise joint at which random nucleotides are added and removed. It is therefore not surprising that the B-cell-receptor (BCR) repertoire of newly generated B cells in the bone marrow of normal individuals contains 50–60% anti-DNA molecules. Less intuitive is the mechanism by which these newly emerging molecules are controlled so that, in normal healthy individuals, DNA-binding antibodies are rare. Weigert presented evidence that mature B cells, that express a BCR heavy chain with an Arg in its antigen-binding site, always express a light chain that is rich in acidic amino acids at the corresponding site, such that the heterodimerization results in a neutral charge. The molecular mechanism involved relies on the continuous rearrangement at the BCR light-chain locus (light-chain editing). It is conceivable that SLE patients are deficient in this editing mechanism, and/or are enriched in Arg-containing receptor genes, so that this process is rendered inefficient. In conclusion, Weigert suggested that more attention should be given to the loci encoding the BCR when defining genetic susceptibility to SLE.

Antigen-mediated activation of B cells is expected to take place in normal lymphoid organs, such as lymph nodes. Consistently, C. Berek (Berlin, Germany) reported that in most RA patients, B cells are activated in conventional lymphoid tissues and after expansion, class switch and somatic hypermutation, migrate preferentially to the inflamed site where Ig secretion by plasma cells is abundant. However, in some patients, functional germinal centres are present in ectopic lymphoid tissues within the synovial tissue. The biological cause of this discrepancy remains to be elucidated and provides another example of the heterogeneity of the biological dysfunctions associated with this disease.

A defect in B-cell development and survival has also been proposed to participate in SLE disorder. The recent characterization

of B-cell-activating factor (BAFF), a survival factor for B cells, supports this hypothesis. Tschopp briefly reviewed the large amount of work that has been conducted on this member of the tumour necrosis factor (TNF) family (Mackay & Kalled, 2002) and, most notably, stated that BAFF levels are increased in various AID patients and that transgenic mice overexpressing BAFF develop a lupus-like syndrome.

Exerting tolerance to control the diverse

Pathological auto-antibody production in AIDs is dependent on the activation of autoreactive helper T cells. Defects in the T-cell compartment, which acts upstream of B-cell activation, is therefore generally believed to have a fundamental role in the development of the disease. Natural tolerance to self-antigens has been long believed to be ensured by 'ignorance of self', be this by active deletion, silencing of autoreactive lymphocytes or antigen seclusion. However, T cells with a potential to cause AIDs are readily detectable in normal healthy individuals. It is now widely accepted that tolerance to self-antigens relies on the regulation and suppression of these autoreactive T cells. The particular subset of T cells that has this function are referred to as 'regulatory T cells' or simply T_{REG} (Bach, 2003; Curotto de Lafaille & Lafaille, 2002).

B. Arnold (Heidelberg, Germany) reported that the trafficking of CD8 T cells through non-lymphoid parenchymal tissues during neonatal life is crucial for the establishment of tolerance to these tissues. He further showed that CD8 T cells rendered tolerant to a tissue-specific antigen that is expressed on parenchymal cells (keratinocytes) act as T_{REG} *in vivo*. These cells inhibit the destructive effector functions of naive CD8 T cells that bear the same antigen specificity. Tolerance induction in this model is strictly restricted to the neonatal period, consistent with Medawar's principle that "self-tolerance is acquired by the developing organism". Arnold proposed that neonatally suppressed autoreactive T_{REG} cells provide a 'memory' of the developing self for the adult immune system.

A large amount of evidence has established that T_{REG} are enriched in a subset of CD4 lymphocytes that express the CD25 marker. S. Hori (Yokohama, Japan) reported that the transcription factor FOXP3, which is mutated in IPEX patients (an AID known as immunodysregulation, polyendocrinopathy, enteropathy, X-linked syndrome), is specifically expressed in $CD4^+CD25^+ T_{REG}$ and is essential for their development and/or function. Collectively, the data recently published on FOXP3 confirm that a defect in the generation of $CD4^+CD25^+ T_{REG}$ is directly associated with severe pathological autoimmunity.

J.F. Bach (Paris, France) discussed the contribution of $CD4^+CD25^+ T_{REG}$ to disease resistance in non-obese diabetes (NOD) mice. He showed that $CD4^+CD25^+$ cells are sufficient to inhibit the diabetes-causing potential of NOD T cells on adoptive transfer into alymphoid NOD-scid (severe combined immunodeficiency) mutant mice. Moreover, NOD mice display a progressive reduction in the number of $CD4^+CD25^+ T_{REG}$, which correlates with onset of diabetes, whereas disease-resistant NOD congenic strains do not. However, natural killer T (NKT) cells also significantly contribute to the prevention of disease development in this model.

Defects in T_{REG} have been rarely associated with antibody-mediated AIDs. Nevertheless, J.J. Lafaille (New York, NY, USA)

showed that T_{REG} control allergic responses, which is an antibody-mediated pathology. Mice that are double-transgenic for a T-cell receptor (TCR) and a BCR of known specificities, but which are deficient in any other lymphocytes, can be induced to develop a hyper-IgE response on simple immunization. This is not the case if, before the immunization, the same animals receive CD4 T cells isolated from wild-type mice. These results show that normal CD4 cells contain T_{REG} that prevent the induced hyper-IgE response. In contrast to what has been reported in other systems, these CD4 T_{REG} need not express CD25 nor do they suppress the initial activation and expansion of effector T cells; they rather inhibit later differentiation into effector/memory T cells. J.D. Isaacs (Newcastle, UK) provided several clues suggesting that RA patients have insufficient numbers of T_{REG} . He reported a T-cell differentiation defect in these patients that is associated with a reduced number of peripheral CD4⁺CD25⁺ T cells (Ponchel *et al.*, 2002). These findings were less evident in patients whose disease was in remission, strengthening the hypothesis that a T_{REG} defect is associated with the disease. Finally, cyclophosphamide, a drug that kills cycling cells, is used to treat both cancer and AID patients. In the latter case, the primary objective is to affect the pathologically activated lymphocytes. However, a direct consequence of this chemotherapy is an important reduction in the number of bone marrow lymphocyte precursors. When compared with unrelated cancer patients, RA patients treated with cyclophosphamide show reduced efficiency in recovering CD4⁺CD45RO⁺ memory T cells, a population known to include CD25⁺ T_{REG} . This defect correlates with a lack of interleukin-7 (IL-7) induction after cessation of the treatment. As IL-7 is a key molecule for lymphocyte development, these results further support the idea that thymopoiesis defects could result in AIDs (Goronzy & Weyand, 2001; Hug *et al.*, 2003), a recurrent theme of the meeting.

A quest for mechanisms

The mechanisms by which T_{REG} exert their regulatory functions remain elusive, if not contradictory. However, it now seems that transforming growth factor (TGF)- β must be a crucial mediator. Bach reported that neutralization of TGF- β by specific antibodies abrogated the protective activity of CD4⁺CD25⁺ T cells in NOD mice. He further subdivided this T_{REG} population according to the level of CD25 expression and revealed that whereas both CD25^{high} and CD25^{low} populations were capable of inhibiting the proliferation of CD25⁻ T cells *in vitro*, only the latter required TGF- β to exert suppression. R.A. Flavell (New Haven, CT, USA) also showed a crucial role for TGF- β in another model of type 1 diabetes, in which pathogenic T cells that express a dominant-negative TGF- β type II receptor (such that TGF- β signalling is blocked) are refractory to the control exerted by CD4⁺CD25⁺ T cells.

J. Demengeot (Oeiras, Portugal) reported that T_{REG} cells selectively migrate into inflamed tissues, where they dampen the recruitment and/or the expansion of effector T cells. Research on the mechanism of lymphocyte migration has revealed the crucial role of chemokines and their receptors (CCRs), and the distinction has been made between constitutively expressed chemokines that are involved in the regulation of homeostasis, and inducible chemokines that are involved in inflammatory reactions. C. Martínez-A (Madrid, Spain) emphasized the complexity of this molecular network. Not only does each receptor

bind to more than one chemokine, but also, in most cases, each chemokine binds to more than one receptor. In addition, whether the receptors are expressed as heterodimers or homodimers affects the threshold of migration induction and therefore the overall kinetics of the downstream events. Martínez-A analysed CCR6 knockout mice and revealed that CD4⁺CD25⁺ cells from these animals, contrary to wild type, prevent neither induced psoriasis-like disease nor inflammatory bowel disease. Further research is expected to increasingly link chemokines with AID, because CCR2^{-/-} mice are more prone to developing asthma and CCR5-deficient animals show early signs of diabetes.

From mechanisms to therapy, it is hoped

With respect to therapy, the expectations are that strategies to increase or re-establish the T_{REG} pool could prevent, ameliorate or even cure AID. Dissecting the mechanisms of T_{REG} maintenance, Lafaille revealed that IL-2 secreted by effector T cells is indispensable for the expansion and/or survival of CD4 T_{REG} cells. Bach showed that the injection of non-mitogenic anti-CD3 monoclonal antibodies, known to cause remission of diabetes in NOD mice, re-establish the functional CD4⁺CD25⁺ T_{REG} pool. Demengeot reported that T_{REG} cells selectively express TLRs that trigger their expansion and effector function on binding to specific ligands such as lipopolysaccharide present on the surface of microbes. This finding may provide a molecular basis for the beneficial effects of bacterial components on the outcome of several AIDs, as clearly shown in the NOD mice and several other models of spontaneous AID. Additional clues were provided by W. van Eden (Utrecht, the Netherlands), who reported that immunization with bacterial heat-shock proteins—some of which are ligands for TLRs—leads to the inhibition of disease development in various experimental models of AID. IL-2 and BCG (the well known vaccine against tuberculosis, composed of bacterial compounds) have been used in the management of AID. Anti-CD3 as well as heat-shock protein 60 (HSP60), the latter in the form of peptides only, are undergoing clinical trials. Now that there is evidence that these compounds are beneficial, because they promote T_{REG} expansion and/or effector function, these broad therapeutical approaches could be developed into more targeted therapies by reducing their undesirable side effects such as general immunosuppression and polyclonal activation.

When the oracle looks at the genes

SLE and RA are complex diseases not only in the usual genetic sense (they are clearly not 'monogenic'), but also because the diagnosis potentially covers a diversity of disease subtypes, and because disease incidence within a genetically homogeneous population (that is, identical twins) is low. For these reasons, genes and/or alleles can only be identified as 'susceptibility' components.

The candidate gene approach is based on an educated guess that links a precise phenotype to the gene products that could potentially mediate this effect. The linkage of the cytotoxic T-lymphocyte antigen 4 (*CTLA4*) gene to AID is the modern prototype of such an approach. First identified as a TCR co-receptor, then as an inhibitor of T-cell activation, the CTLA4 gene product was later proposed to be required for CD4 T_{REG} function. It is now established that in humans, particular alleles of the *CTLA4* gene associate with a large number of AIDs. These include RA, Graves disease, type 1 diabetes, MS and, finally, SLE as presented in a

poster by M. Barreto (Oeiras, Portugal). Studies on programmed death 1 (PD1), another T-cell co-receptor that negatively regulates TCR signalling, show our ignorance on what is vaguely referred to as 'genetic background'. T. Okazaki (Kyoto, Japan) reminded us that mice deficient in PD1 develop severe autoimmunity, but the tissues that are affected vary according to the genetic background. Thus, C57Bl/6 mice develop glomerulonephritis and arthritis, whereas BALB/c develop antibody-mediated cardiomyopathy. In a related approach, F. Sanchez-Madrid (Madrid, Spain) reported that CD69, which is expressed on the surface of activated lymphocytes, modulates inflammation in a model of induced arthritis in mice.

Forward genetics is now empowered by the new tools of genomics, such as automated sequencing, mapping, single-nucleotide polymorphism (SNP) analyses, gene expression profiling and multi-parametric databases. G. Peltz (Palo Alto, CA, USA) reported on the analysis of a murine genetic model of T-helper-cell differentiation, which led to the identification of transcription factor 7 (*TCF7*) as the genetic locus that regulates Th1/2 differentiation. Furthermore, analysis of polymorphisms in the human *TCF7* gene identified alleles that are associated with susceptibility to human atopic allergy and type 1 diabetes. He also presented a haplotype-based computational prediction method for the analysis of complex traits in mice. Haplotype maps were prepared from a murine database of SNPs, which was previously used for *in silico* prediction of the chromosomal regions that regulate a given phenotypic trait (Grupe *et al.*, 2001). The use of the haplotype-based predictions increased the accuracy and precision of the predictions. The hope that genomics will help to explain the causes of autoimmunity was therefore greatly strengthened.

What matters most: therapeutic strategies

To pinpoint the key biological events that can be targeted by therapies, W. Haas (Oeiras, Portugal) presented a simplified scheme of the chain of events leading to chronic inflammatory diseases and integrated several of the points of discussion that emerged during the meeting. An adaptation of this scheme is presented in Fig. 1. Therapies that aim to prevent the occurrence of the disease, lower the manifestation of the inflammation or prevent relapses should be distinguished because the cellular targets are different. Haas further presented an overview of the present treatments for AIDs and the ongoing clinical trials. The take-home message was, sadly, that there is still no cure available for SLE or RA, and that clinicians are limited to managing the disease and improving the quality of life of their patients.

In addition, as pointed out by A. Tyndall (Basel, Switzerland), the success of therapy depends on early diagnosis so that it can be applied before organs are damaged. The possibility of early diagnosis would greatly benefit from previous genotyping to identify people at high risk and should be based on parameters that measure the dysregulation of the immune system itself and not the target tissues. Moreover, such surrogates are needed for the follow up of treatments because amelioration of the clinical condition could be delayed by more than six weeks (J.D. Isaacs). Finally, Isaacs also pointed out that responses to each therapeutic protocol vary from one patient to another in a rather unpredictable way, indicating once more our ignorance of the subtypes of the diseases.

An overview of B-cell homeostasis presented by A. Freitas (Paris, France), together with the diverse interventions that point

Fig. 1 | Main events leading to chronic inflammatory autoimmune disease.

A yet to be understood event X, conditioned by genetic and environmental factors, leads to the activation of self-reactive T lymphocytes by antigen-presenting cells (APCs). In normal individuals, this is prevented by regulatory T cells (T_{REG}). In turn, the effector T cells (T_E) activate B lymphocytes and infiltrate target tissues. These two stages of the immune response seem also to be controlled by T_{REG}. A cascade of events, involving leukocyte infiltration, cytokine production, complement activation, and production of antibodies and prostanoids leads to tissue damage. Eventually, resolution of the inflammatory reaction that results from several regulatory mechanisms of both the innate and the adaptive parts of the immune system leads to a phase of remission. Relapses can occur due to the activation of memory T cells (T_M) by self-antigens that are released from damaged tissue. As long as event X remains unknown, therapeutic strategies must aim at inhibiting (red blocked arrows) ongoing inflammatory reactions and/or enhancing (green arrow) T_{REG} function. Preventative therapies should enhance T_{REG} function, whereas curative therapies should aim at the prevention of relapses.

to the necessity for the immune system to be intact for it to function, lead to a clearer view of what constitutes a promising therapeutic strategy. It was concluded that a successful curative approach needs to reset correctly the immune system as a whole—that is, the chemical reduction or elimination of the pathological lymphocytes should be followed by the restoration of a fully diversified antigen-receptor repertoire (BCR and TCR) and, eventually, of the T_{REG} pool. Therefore, provided the diagnosis is early, the therapy of the future may well be chemically induced aplasia followed by autologous haematopoietic stem-cell transfer (A. Tyndall). In such a scenario, the chemotherapy is designed to kill all haematopoietic precursors and the patient becomes devoid of lymphocytes. Before the treatment, however, haematopoietic stem cells are isolated and stored until total aplasia has been reached, and at this point, they are re-injected. Such protocols are under prospective trials for scleroderma, MS, RA and SLE, and all of these are being coordinated by the European Group for Blood and Marrow Transplantation (www.EBMT.org). However, even though these approaches are promising, it is less than five years since the first attempt and it is certainly too early to evaluate their curative properties.

In summary, the hope is high that genetic approaches will help to establish early diagnosis of SLE and RA, so that patients can be treated before tissues are irreversibly damaged. A lot is expected from the recent progress in understanding the mechanisms of regulation of the (auto)immune responses: invasive therapies aimed at silencing the 'bad' components of the immune system will be complemented with approaches aimed at stimulating the 'good'. Importantly, it seems that the field of immunology has reached a point at which the components of the immune system have been sufficiently analysed so that this knowledge can now be integrated. Thus, the agenda of immunology is now to understand the immune system for what it is: a complex system of interactive components. Understanding complex diseases such as SLE and RA, the pathology of which results from a cascade of events involving many cellular components of the immune system, constitutes a challenge that must be met. This is especially true now that clinicians and fundamental biologists have found a common language, and share knowledge, which guarantees that rational therapies will rapidly replace empirical approaches to treatment.

REFERENCES

- Bach, J.F. (2003) Regulatory T cells under scrutiny. *Nature Rev. Immunol.*, **3**, 189–198.
- Curotto de Lafaille, M.A. & Lafaille, J.J. (2002) CD4(+) regulatory T cells in autoimmunity and allergy. *Curr. Opin. Immunol.*, **14**, 771–778.
- Goronzy, J.J. & Weyand, C.M. (2001) Thymic function and peripheral T-cell homeostasis in rheumatoid arthritis. *Trends Immunol.*, **22**, 251–255.
- Grupe, A., Germer, S., Usuka, J., Aud, D., Belknap, J.K., Klein, R.F., Ahluwalia, M.K., Higuchi, R. & Peltz, G. (2001) *In silico* mapping of complex disease-related traits in mice. *Science*, **292**, 1915–1918.
- Hug, A., Korpö, M., Schröder, I., Haas, J., Glatz, K., Storch-Hagenlocher, B. & Wildemann, B. (2003) Thymic export function and T-cell homeostasis in patients with relapsing remitting multiple sclerosis. *J. Immunol.*, **171**, 432–437.
- Kim, S.J., Gershov, D., Ma, X., Brot, N. & Elkon, K.B. (2003) Opsonization of apoptotic cells and its effect on macrophage and T cell immune responses. *Ann. NY Acad. Sci.*, **987**, 68–78.
- Mackay, F. & Kallied, S.L. (2002) TNF ligands and receptors in autoimmunity: an update. *Curr. Opin. Immunol.*, **14**, 783–790.
- Ponchel, F. *et al.* (2002) Dysregulated lymphocyte proliferation and differentiation in patients with rheumatoid arthritis. *Blood*, **100**, 4550–4556.
- Tschopp, J., Martinon, F. & Burns, K. (2003) NALPs: a novel protein family involved in inflammation. *Nature Rev. Mol. Cell Biol.*, **4**, 95–104.

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