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Disregulation of the Immune System Leading to Autoimmunity

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Introduction

Recent work in our laboratory on the subjects of containment of the functional T-cell receptor repertoire, protective MHC genes, T-cell cytokines in arthritis, and oral tolerance, is described. Together they make a powerful case for control of autoimmune disease by cytokine balance.

The general properties of B and T cells indicate that B cells would need to operate proportional representation in order of their affinity-selection to take place. Whether T cells would operate the opposite system (i.e. first past the post) has been less clear, and it is to this question that the following study is addressed.

T-hybridoma Analysis of the Response of Mice to F Liver Protein

The analysis has been carried out by Susanne Schneider on the response of mice to mouse F liver protein. This protein occurs in two forms, so that mice of one allotype can make a sizeable antibody response to protein of the other allotype, provided that (i) the protein is administered in adjuvant, and (ii) they have the responder MHC allele H-2A^k. This response is of particular interest for the following three reasons: (i) the protein does not leak out of the liver in quantities sufficient to tolerise B cells, so that the antibody response has a high signal-to-noise ratio; (ii) the protein is immunologically noisy (presumably other liver proteins also have allotypic variants, but none have been found to signal in the way that F protein does), (iii) the protein belongs to the select but growing list of antigens which have in H-2A^b a down-regulatory (immunosuppressive) MHC allele. This is a system which we have had under study for many years (Mitchison & Simon 1990, Mitchinson 1993).

The two most significant findings in this analysis were as follows: First, mice do not respond to immunization with self-F protein. Nevertheless they must have T cells able to recognise self-F protein present with their immune system, as T-hybridomas able to do so occur in hybridoma collections made from mice immunised with either self- or allo-F protein. These hybridomas are of course less frequent than ones which recognise the allo protein. They could be characterised by means of peptides spanning the allo/self substitution at position 101 in the protein, where their affinity for the self-peptide was always less than for the allo-peptide. Their presence came at first as a surprise, but it was not to be the only one. Continuing the study, mice were treated from birth with polyclonal antibody to F protein. This might have been expected to prevent tolerance-induction, or at least to lower the affinity of the T cell response. What happened was that the treatment had no detectable effect on the affinity of the hybridomas recognising self-peptide, although it did clearly increase their frequency.

At that point we turned back to the old literature on B cells, and noticed how differently these T cells were behaving, particularly towards passively administered antibody. But at the same time we noticed how little affinity seemed to matter throughout the T cell literature, in comparison with frequency and activation (Lightstone et al.1993). So that is where we stand at present, stout supporters of the "first past the post" view of the behaviour of T cells.

Suppression Mediated by H-2A^b

Following up the protective effect of MHC class II genes frequently observed in immunological diseases in man, we began a hunt for any such genes able to protect mice against collagen-induced arthritis. The idea was that this might pick up all sorts of down-regulatory effects. The hunt was carried out by crossing the susceptibility gene for this disease, H-2Aq, to a panel of potentially down-regulatory H-2 haplotypes. This was easy to do in the mouse, because of the wealth of recombinants available within the H-2 region. We eventually identified the H-2A^b gene as clearly able to protect against this form of arthritis.

Without going into detail, our results so far can be summarised as follows. The average arthritis score obtained with bovine types II collagen was significantly and reproducibly lower in two H-2A^b hybrids (DBA/1 × B10. DBA/1 × B10.A(5R) than in three non-H-2A^b ones DBA/1 × B10.A, DBA/1 × B10.A(4R), DBA/1 × B10.MBR). None of the other MHC substitutions examined had any detectable effect (at H-2E and H-2D). It is particularly significant that hybrids expressing H-2E were not detectably protected, because this MHC molecule has been reported to have an immunosuppressive effect in several other systems.

In addition, a lower incidence and briefer duration of arthritis was observed in the H-2A^b than in the non-H-2A^b mice.

The identification of H-2A^b came as no surprise, as the same gene had previously been found to down-regulate several other immune responses, involving at least three other apparently quite unrelated antigens.

What could this mean? The phenomenon of bunching argues against an epitope-specific mechanism, such as the epitope-affinity hierarchy which has been proposed to account for the protective effect of HLA DR2 in type I diabetes. We think it likely that H-2A^b-restricted T cells are generally biassed towards production of one or more immunoinhibitory cytokine, although how and why this should occur we do not know. Perhaps the bias is introduced through priming by one or more environmental antigen, with which the collagen cross-reacts. This idea resurrects the old theory of antigenic mimicry as a cause of autoimmunity, under a new guise.

Quantitative Analysis of Cytokine mRNA

To learn more about how these inhibitory MHC genes might work, we felt a need to measure cytokine production as accurately as possible. To that end Monika Brunner has established quantitative PCR for mouse cytokine mRNA in our laboratory, which she is now applying to responses inhibited by H-2A^b. The first response chosen for analysis was to allo-F-liver protein, as described above, because (i) the T cell response is exceptionally strong, as judged by early swelling of the draining lymph node, and (ii) the inhibitory effect is also strong.

Thus far the analysis shows that levels of mRNA for the Thl cytokines IL-2 and IFNγ and the inhibitory cytokine TGFβ, but not the Th2 cytokine IL-4, are high in H-2A^b mice.

We regard these data as strongly supporting the possibility that this protective gene operates via transcriptional control of cytokine genes. We are none the wiser about how what evokes that control, and are left

with the speculation suggested above, that priming by one more environmental antigens may bias cytokine activity in the H-2A^b-restricted T cell subset.

T-cell Cytokines in Arthritis

In spite of many negative reports, my colleagues Joachim Sieper, Katharina Simon, and Eva Seipelt decided to look again for T cell cytokines in joints, using the most sensitive methods available. They chose to use two methods of detecting cytokine mRNA in the hope that they would check against one another, in situ hybridisation (i.e. detecting mRNA in individual T cells) and the polymerase chain reaction. They chose also to examine two forms of inflammatory arthritis, in the hope that they might yield contrasting results. Both hopes have been fulfilled. In a nutshell, rheumatoid arthritis emerges from this study as predominantly a Th1 disease, while reactive arthritis emerges as a Th2 ones. IFNγ and IL-10 are transcribed to a detectable extent in most affected synovia in both diseases, and IL-2 in some synovia in both. The key finding is that IL-4 appears regularly in reactive but not in rheumatoid arthritis.

Whether a clinical correlation of this kind reflects a causal relationship can be established only by intervention. Possible methods of perturbing the cytokine balance include such direct forms of treatment as implanting cytokine genes or giving anti-cytokine antibodies, or indirect ones such as giving antigens or cytokine-selective drugs.

Oral Tolerance

We regard oral tolerance as an excellent example of a form of treatment that may indirectly perturb the cytokine profile, possibly via localised production of TGFβ. Clinical trials carried out in Boston by H Weiner and colleagues have excited much interest in this form of intervention. In collaboration with Joachim Sieper, and with support from the BMFT, we are organising a Berlin-wide trial of oral tolerance of bovine type II collagen in rheumatoid arthritis, which will include for the first time a (limited) dose-response study.

An Assessment of the Importance of T-cell Cytokines in Rheumatic Disease

A number of difficulties need to be considered before going overboard for the possibility of the balance of T-cell cytokines determining the outcome of inflammatory rheumatic diseases. T cells are not found in large numbers in the inflamed rheumatoid arthritis joint, and few of them produce detectable levels of cytokines (Firestein & Zvaifler 1990); however, there is some comfort in the fact that few T cells in peripheral blood do so either, even after activation, and that few cells do so at any one time even within "cytokine-positive" T-cell clones. Furthermore, anti-T-cell therapy with monoclonal antibodies has proved only moderately and sporadically effective, in comparison with the dramatic effects claimed for monoclonal antibody directed at the inflammatory cytokine TNF which is produced mainly by macrophage. This has led eminent rheumatologists to postulate a self-sustaining loop (autocrine/paracrine loop) established between macrophage and synovial fibroblast (Firestein & Zvaifler 1990, 1993 and Winchester et al. 1993), with no more than a nebulous guiding role assigned to T cells. A somewhat similar loop has been postulated for scleroderma, between fiboblasts, the extra-cellular matrix, and PDGF (Ivarsson et al. 1993). A significant point is that the "activated state" of synoviocytes can be passaged by bulk populations of cells, but not by single cells. This raises a question about how the loop could first be established.

For the time being a guiding role for T cells remains very much a matter of faith. So far

even in the best characterised Th1/Th2 diseases, including leprosy and to a lesser extent parasitic infections and allergy, the case for a decisive role for T-cell cytokine balance rests almost solely on clinical correlations. We must still wait for the decisive evidence likely to emerge from cytokine and anti-cytokine therapy.

The Future of Rheumatology

The quest for therapy will surely require ever more sophisticated animal models. With that thought in mind we close with figure 1, which depicts the ultimate research weapon. This is not meant only as a joke; cytokine gene therapy looms ever larger in the future of rheumatology.

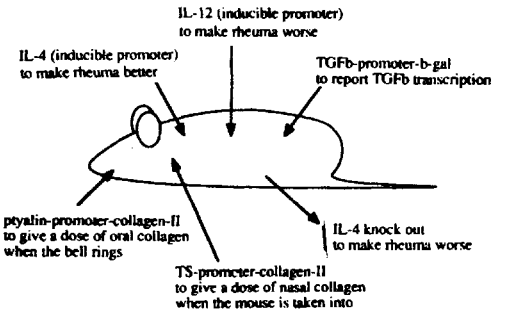

Figure 1 The ultimate rheumatism research mouse

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