EDITORIAL REVIEW

The role of cellular immunity in systemic vasculitis

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INTRODUCTION

Our knowledge of the cellular component of autoimmunity (autoreactive T cells) lags far behind our knowledge of the humoral component (autoantibodies). With some rare exceptions (e.g. autonomous expansion of an autoantibody-producing B cell clone) it is probable that T cells are involved at least indirectly in providing help for the production of the better characterized autoantibodies. Although autoreactive T cells have been identified in some diseases [1–3], evidence for a direct role in pathogenesis is difficult to obtain in man.

The development of knowledge concerning autoreactivity in systemic vasculitis (SV) is proceeding along familiar lines. These diseases were long suspected to have an autoimmune basis, and this suspicion was strengthened by the description of anti-neutrophil cytoplasmic antibodies (ANCA) [4]. ANCA are highly sensitive and specific for active primary systemic vasculitides involving small blood vessels (Wegener's granulomatosis (WG), microscopic polyangiitis) [5]. As well as their undoubted clinical utility it is possible that ANCA also play a role in pathogenesis [6]. Following this description of humoral autoreactivity in SV, it was natural to ask whether autoreactive T cells were also present.

REASONS FOR IMPLICATING T CELLS IN SV

Since ANCA tend to be high-affinity IgG antibodies, including a bias towards the IgG4 subclass [7], T cell help is implied. This, together with the lack of immunoglobulin deposition at the sites of injury and the frequent presence of T cells in these areas, sometimes with granuloma formation [8–10], has led investigators to suspect a role for cellular immunity in the pathogenesis of SV [11]. Markers of T cell activation are elevated in the circulation of some patients with active disease [12,13]. Drugs such as cyclosporin, whose main action is against T cells, may have useful therapeutic effects in some patients [14], and MoAbs against lymphocytes also show promise in the treatment of refractory disease [15,16]. Thus there is much indirect evidence to implicate T cells in the pathogenesis of SV, and considerable research effort has been applied in recent years to attempts to directly demonstrate autoreactive T cells against neutrophil antigens.

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IN VITRO ANALYSIS OF T CELLS FROM PATIENTS WITH SV

Two early reports, on small numbers of subjects, suggested that T cell proliferative responses to neutrophil antigens could be demonstrated in patients with SV, but not in controls [17,18]. By contrast, in our own study using a crude extract of neutrophil cytoplasm as the target antigen, although we did see low levels of T cell proliferation in some of the 36 patients we studied, similar low levels of proliferation were seen in controls. There was no significant difference between patient and control groups [19]. The crude nature of the stimulating antigen could explain a degree of non-specific mitogenicity, and we also speculated that a mixture of stimulatory and inhibitory antigens could be present, cancelling each other out [19]. This problem was addressed by Brouwer *et al.* in a recent paper published in this journal [20]. These authors used purified preparations of proteinase 3 (PR3) and myeloperoxidase (MPO), the major antigenic targets of ANCA, in lymphocyte proliferation assays. MPO markedly inhibited non-antigen-specific lymphocyte proliferative responses; this effect could be prevented by heat-inactivation of the MPO. There was no difference in proliferative responses to heat-inactivated MPO between patients (with anti-MPO ANCA) and controls: low levels of proliferation were seen in both groups. With PR3, again low levels of proliferation were seen in both patients and controls: heat-inactivation of the PR3 slightly reduced the magnitude of response in both groups, indicating some minor mitogenic effect. At one of the four concentrations of PR3 tested, the authors report a significant difference between the proliferative responses in patients *versus* controls, but the P value was not apparently adjusted to reflect the fact that four independent comparisons were being made [21]. At all concentrations there was considerable overlap between the two groups.

A paper in this edition of CEI [22], from one of the groups who initially reported positive results [17], also describes the use of purified PR3 in lymphocyte proliferation assays [22]. There was again a minor mitogenic effect; there was no difference in the mean stimulation index in response to PR3 between patients and controls. Using a crude extract of neutrophil azurophilic granules there was a slight inhibitory effect, and a trend towards higher reactivity in patients, but this did not achieve statistical significance, and again there was overlap between patients and controls. Although only patients were judged to be positive in these assays, χ^2 analysis shows that, for the numbers reported, this frequency of positivity was not

significantly different from the control group. The other group which reported encouraging initial results [18] have as yet been unable to reproduce their results using purified antigens (N. Rasmussen, personal communication).

Thus we would contend that there is to date little or no convincing direct evidence of significant T cell autoreactivity in SV. Despite careful efforts to avoid inhibitory or mitogenic effects of the various antigen preparations, no clear difference has been demonstrated in any of the *in vitro* studies between patients and controls.

ALTERNATIVE APPROACHES

How might the important question of T cell reactivity in these diseases be further addressed? It seems unlikely that variations in the degree of disease activity can explain the negative findings. The patients studied by Brouwer *et al.* [20] all had inactive disease; our study [19] was of patients with active disease prior to immunosuppression. In the study by Ballieux *et al.* [22] there were no differences between patients with active *versus* inactive disease. One approach is to use T cells derived from involved tissues rather than peripheral blood cells. Obviously, only small numbers of cells are likely to be available from biopsy material. However, such cells can be non-specifically expanded for *in vitro* analysis, or analysed using sensitive molecular techniques for (e.g.) T cell receptor gene usage. Ballieux *et al.* [22] tested T cell lines derived from tissue biopsies from patients with WG for cytotoxic T cell activity against cells bearing PR3 peptides bound to an appropriate MHC class I molecule. Unfortunately no cytolytic activity could be demonstrated.

ARE T CELLS INVOLVED AT ALL?

Thus, extensive *in vitro* analysis has failed to provide convincing evidence for the existence of autoreactive T cells against neutrophil antigens in patients with SV. Animal models of SV are in general somewhat unsatisfactory [23], and it seems likely that further direct testing of the importance of cellular immunity in these diseases will have to await the development and clinical usage of anti-T cell reagents of greater specificity than those currently available. The possibility must be considered that T cells are not directly involved. The lack of consistent association between SV and any particular MHC alleles can be taken as indirect evidence that this may indeed be the case. T cells of course recognize their epitopes in the context of products of the MHC. A breakdown in self-tolerance at the T cell level is likely to be limited to a very restricted set of epitopes. The ability of particular allelic MHC products to bind and present these epitopes, or to so shape the developing T cell repertoire as to allow their recognition, are the assumed mechanisms underlying the well known association between many autoimmune diseases and particular MHC alleles. Studies of the distribution of MHC alleles in patients with systemic vasculitis have not produced consistent results (reviewed in [24]), and the lack of an association with MHC class II alleles in the largest study to date [24] suggests that any such association is weak, if it exists at all. One possible interpretation of this finding is that the putative T cells involved in providing help for ANCA production are directed against exogenous antigens. The multiplicity of foreign epitopes available on, for instance,

bacteria will obscure any MHC associations. The well recognized link between infection and exacerbation of vasculitis [25,26], and the occasional response to antibacterial treatment alone [27], may be relevant in this context. This speculative possibility requires some physical linkage between bacteria and the ANCA autoantigens in order for the T cell to provide effective help, but such intermolecular intrastructural linkage is well recognized [28]. Could neutrophils containing phagocytosed bacteria provide the necessary link? This possibility suggests that another explanation for the failure to demonstrate T cell autoreactivity in the studies so far could be that autoreactive T cells in SV may not be recognizing ANCA autoantigens, so that the antigen preparations used have been inappropriate.

CONCLUSION

Mechanisms whereby the ANCA autoantibodies themselves may cause tissue injury have been proposed [6], but remain unproven. Whilst we firmly believe that SV has an autoimmune etiology, detailed knowledge of the precise cellular mechanisms involved continues to elude clinicians and scientists alike.

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