Leading article

Why study T cell subsets in Crohn’s disease?

Investigations of immunology in inflammatory bowel disease have always presented problems. The primary disease processes are unknown and when the epithelial barrier of the gut has been breached, many local inflammatory effects in the tissues are likely to result from access of bacteria and other substances. Furthermore, nutritional status, antibiotics, immunosuppressive drugs, dietary manipulations, and the surgical complications of disease are all likely to affect immunity. Thus in Crohn’s disease, no cohesive pattern of results has emerged despite a considerable literature on humoral and cell mediated immunity. Most of these studies, however, have concerned systemic immune responses such as serum immunoglobulins, serum antibody titres and the properties of peripheral blood lymphocytes.

In recent years work in experimental animals has shown, unequivocally, that the gut associated lymphoid tissues are distinct from the systemic immune apparatus in many respects – cellular constituents, effector functions, and the regulation of these functions. The intestinal mucosa provides a major interface between the tissues of the body and the antigens of the environment, most of which are harmless. Thus in contrast with a need for vigorous and active immune responses to parenterally encountered antigens in the systemic immune system, there is a biological necessity for inhibition of most immune responses at gut level. Normally, when antigen is presented to the body via the gastrointestinal tract there is a local secretory IgA antibody response and induction of a state of hyporesponsiveness – oral tolerance – so that there is antigen-specific suppression of subsequent systemic antibody and cell mediated immune (CMI) responses.1, 2 Effector CMI responses in the gut mucosa are also absent if antigen has first been encountered via the gut.3 Yet in addition to this orderly pattern of harmless, low grade immune responses to enteric antigens, it is necessary that the individual retains a capacity to mount protective immune responses to enteric pathogens. In view of the so called ‘innocent bystander’ effect of local hypersensitivity, particularly involving IgE antibodies and cell mediated immunity, immune mediated damage to the gut will often occur in the course of such immune responses in infectious diseases, even in normal individuals.4 Such effects will be transient if the antigen is rapidly eliminated by the immune response. Inappropriate enteropathetic immune reactions also occur. In some instances the antigen concerned is recognised, for example in cows’ milk protein sensitive enteropathy with malabsorption, but in the so called idiopathic inflammatory bowel diseases, no relevant antigens have yet been identified. An attractive theory of pathogenesis of these diseases has therefore emerged, which states that in some way the usual constraints on local immune reactions have failed. T lymphocytes and their products are
now known to be the principal agents which regulate immune responses. Thus to test this theory, the nature and properties of intestinal T lymphocytes in Crohn’s disease and ulcerative colitis must be re-examined.

Despite the morphological similarity of all lymphocytes there is considerable heterogeneity with respect to their origins, lifespan, mobility, and functions. The two main categories are T (thymus dependent) and B (thymus independent, bone marrow derived) cells. Whereas B lymphocytes are the precursors of plasma cells and are necessary for humoral immunity, the roles of T lymphocytes are more complex. T cells have the capacity to recognise specific antigens, to execute unique effector functions and to regulate, by help and suppression, the functions of other T cells, B cells, non B, non T (null) cells and macrophages.6 Effector mechanisms and immunoregulation are mediated not only by cell contact, but also by soluble products of T cells. Not all T cells can perform these functions. In vivo and in vitro investigations have shown two main T cell subpopulations, T helper and T suppressor.

In 1969, Boyse and his colleagues reported the existence of a series of differentiation antigens which were present in the cell membrane of mouse T lymphocytes.6 In peripheral blood, the T cell subpopulations which had already been recognised as having different functions, were also found to possess different membrane antigens. The Lyt 1+23– cells acted as T helper cells for both B cell responses and T cell responses, whereas Lyt 1–23+ cells were either T suppressor or cytotoxic T cells. Much further work has shown that although these are the two principal subsets of mouse T lymphocytes, many functionally distinct T cell subpopulations can be found and there is a broad correlation between the functional properties of the given T cell population, and its surface antigen phenotype.7 Many in vivo and in vitro experiments, now supplemented by investigations of T cell clones, are revealing complex interactions between cells in the generation and regulation of all immune responses. The limited experiments which have concerned immune responses to fed antigen tend to show good correlation between immunoregulatory T cell properties and the observed in vivo responses of the gut, in that feeding antigen leads to dual activation of helper cells for the IgA system and suppressor cells for IgM and IgG synthesis.8 9 There is also evidence of induction of suppressor cells which regulate mucosal CMI.3

Human T cells also possess cell surface antigens, which have been defined by using heteroantisera, autoantibodies, and monoclonal antibodies.10 11 In the literature of the 1970s, many different codes and abbreviations were used to describe these antigens and associated antibodies. Fortunately the widespread use of monoclonal antibodies, interchange of reagents between groups and a recent international workshop on human leucocyte differentiation antigens (held in Paris in November 1982, proceedings to be published by Springer Verlag) have led to the acceptance of a simple nomenclature for the antigens in the surface membrane of human T lymphocytes – T1, T3, T4 etc. Lymphocytes gain and lose various cell markers, including T6, T9 and T10 antigens, as they differentiate and mature within the thymus. These markers have been used to examine T cell maturation in immunodeficiency diseases and to classify T cell malignancies. The mature T cells, circulating in peripheral blood, possess T1 and T3 antigens. In addition the T4 antigen is expressed on
approximately 60% and T8 on some 20% of peripheral T cells. The T4 and T8 positive subsets correspond to helper and suppressor lymphocyte populations, previously defined by using heteroantisera. Abnormalities of immunoregulatory T cell numbers have been shown to correspond with in vitro tests of T cell functions, and clinical disease in patients with hypogammaglobulinaemia or graft-versus-host reaction. In addition, more subtle alterations in absolute and relative numbers of T4 and T8 bearing lymphocytes are being reported in many inflammatory, allergic and infectious diseases.

Cell surface antigens in tissue lymphocytes can be examined either by using cell suspensions, or by application of immunofluorescence and immunoenzyme techniques to tissue sections. For example, T cell subsets have been examined in lesions of leprosy, to test the theory that the rapid advance of the lesions in lepromatous leprosy results from a failure of cell mediated immunity, which itself results from abnormal immunoregulation in the host. The patterns of T cell subset infiltrate appear to correlate with the local tissue immune responses in this disease. Tuberculoid infiltrates, in which a vigorous local immune response is present, contain a substantial proportion of T cells which are T4 positive – that is, of the helper/inducer phenotype. In the lepromatous form, a much higher proportion of T cells are T8 positive, corresponding with the suppressor phenotype. Patterns of T4 and T8 cell infiltrate have also been found to correlate with states of enhanced or suppressed immunity in diseases of skin, joints, lung, liver, and kidney.

There are many lymphocytes in the gut mucosa and most of these are thymus dependent T cells. Selby and his colleagues have described, in a series of papers, the normal distribution of T cell subsets within the human intestinal mucosa – T8 positive cells predominating within the epithelium, T4 cells in the lamina propria. They found an excess of T8 positive cells in coeliac disease, but in Crohn’s disease and ulcerative colitis T4 and T8 cell numbers were similar to those in normal bowel mucosa – perhaps disappointing findings in the context of the hypothesis of abnormal immunoregulation described above. Nonetheless, it will be important to investigate function as well as surface antigen characteristics of the lymphocytes infiltrating the gut in inflammatory and other bowel diseases.

Suspensions of lymphocytes can be prepared from lymph, peripheral blood, lymph nodes or tissues. Several techniques have been described, for isolation of lymphoid cells from the gut mucosa. A number of difficulties arise, however, when such suspensions are being used to investigate properties of gut lymphoid cell suspensions, particularly in diseased subjects. The method by which lymphocytes are separated from epithelium and other non-lymphoid cells is likely to deplete some types of lymphocyte more than others – for example, those which are more readily adherent. As separation techniques may take up to 24 hours, some short-lived cells are unlikely to survive the initial preparatory process. Techniques which enrich either for intraepithelial or lamina propria lymphocytes will give completely different results, because of the quite separate natures of lymphoid cells which inhabit these two microenvironments. Finally, assay systems which have been developed and evaluated for peripheral blood lymphocytes or even lymph node cell suspensions need complete reappraisal when used for investigation of tissue lymphocytes. Despite all
these problems the results reported in this issue of Gut by Fiocchi and his colleagues are very convincing and illustrate how many of the problems outlined above may be overcome. They prepared suspensions of lamina propria lymphocytes from bowel which had been resected either from patients with inflammatory bowel disease or non-inflammatory diseases such as colon cancer. They examined the capacity of gut mucosal lymphocytes to regulate the functions of the patient's own peripheral blood lymphocytes in a standard immunological assay, phytohaemagglutin induced blast transformation. Cell marker studies showed that the lymphocytes prepared from patients with inflammatory bowel disease contained similar proportions of T and B cells and macrophages, as those prepared from controls. Unequivocal functional differences between the two groups were demonstrated. The mucosal lymphocytes from patients with inflammatory bowel disease had strikingly enhanced suppressor cell activity in the autologous system used. It remains to be shown whether a similar degree of suppression by gut mucosal lymphocytes can be shown in other T cell systems and in regulation of B cell function, and whether such suppressor activity is capable of further modulation by intraepithelial or circulating lymphocytes. In the present state of knowledge it is really counter productive to speculate on possible mechanisms responsible for the altered immunoregulatory function within this population. Whether the ultimate in vivo effects are beneficial or immunopathological, remains to be discovered.

The aims of research in intestinal immunoregulation must, ultimately, be threefold. The nature of infiltrating lymphoid cells can be defined by studies of cell surface antigens, complemented by descriptions of morphology. A wide range of in vitro techniques must be used to examine their functions. In addition methods must be developed to allow us to examine in vivo the immunoregulatory activity of the gut associated lymphoid tissues, for example by studies of induction of active immune responses and tolerance to enterically applied antigens. Development of techniques and interpretation of the results in man would be greatly facilitated if there were satisfactory animal models of chronic inflammatory bowel disease.

In contrast with the striking advances in some other aspects of clinical immunology, developments in knowledge of intestinal immune functions in man have been slow and patchy. The principal reason for this is likely to be that direct access to the intestinal lymphoid system of man still poses major practical problems. Yet these must be faced, because it is clear that investigations of immunity, based on studies of peripheral blood, have little relevance to events taking place within the gastrointestinal tract.

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References

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