

Lymphocyte subsets in hypersensitivity pneumonitis

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Hypersensitivity pneumonitis (extrinsic allergic alveolitis) is the pulmonary manifestation of hypersensitivity to various categories of inhaled organic antigens. These include animal proteins from the faeces of pigeons or parakeets, or from duck and turkey feathers and antigens from various plant sources such as mouldy hay, sugar cane, bark and mushrooms. Certain drugs may also produce this reaction [1-3].

Sensitized individuals exposed to a large challenge develop an acute febrile illness, characterized by the abrupt onset of breathlessness, cough and chest pain within 4-6 h after exposure. This acute hypersensitivity reaction usually resolves spontaneously without sequelae after the offending agent has been withdrawn. Chronic insidious exposure to low levels of antigen, however, may lead to persistent attacks of breathlessness on exertion, with or without previous acute attacks, and diffuse interstitial lung disease, which in some cases will progress to interstitial fibrosis [4-5]. Histological studies of lung biopsies have shown that the inflammatory process affects the interstitium, alveolar space and terminal airways in an uneven distribution in the lung biopsy [6]. Epithelioid granulomas containing multinucleated histiocytes are often, but not invariably, present and are usually distributed around distal airways, in the interstitium and within alveolar spaces [6, 7].

The pathogenesis of hypersensitivity pneumonitis is not yet fully understood but immune mechanisms with or without amplification by immune complexes, lymphokines and other biological modifiers are thought to be involved [2]. Repeated antigen exposure in a susceptible host appears to stimulate chemotaxis and sensitize T-lymphocytes and macrophages, resulting in granuloma formation. Continued exposure finally results in the elaboration of growth factors that stimulate fibroblasts and result in collagen production [8].

Our understanding of the pathogenesis of this disease has been improved recently by widespread use of bronchoalveolar lavage (BAL). This provides a relatively non-invasive technique for sampling cells from peripheral lung tissue and, using monoclonal antibodies, cells recovered by BAL can be studied both from a phenotypic and functional point of view. The total number of cells recovered by BAL from patients with hypersensitivity pneumonitis is greatly increased, being three to five times that observed from controls

[9, 10]. A large part of this increase can be accounted for by lymphocytes [9], although an influx of neutrophils into the lungs has been observed soon after antigen challenge [10, 11]. Using monoclonal antibodies, a number of workers have demonstrated that most of the BAL lymphocytes expressed T-cell markers, while only a small proportion expressed B-cell related markers [9, 12, 13]. Analyses of T-cell subsets from these patients have shown that CD8+ lymphocytes are the predominant cells with a resulting inversion of the CD4:CD8 ratio [14-17] and similar observations have been made in animal models [18, 19]. Furthermore, the cells found in BAL from patients with hypersensitivity pneumonitis reflect those present in lung tissue of patients with pigeon breeders' disease [20], while peripheral blood T-cells remain normal [16].

The timing of BAL or biopsy specimens related to antigen exposure affects the preponderance of cells. TRENTIN *et al.* [14] found a persistent increase of CD8+ cells and reversal of the CD4:CD8 ratio in patients who continued to be regularly exposed to specific antigens at work. A persistent increase in the population of natural killer (NK) cells has also been demonstrated in these patients [14, 21]. Other patients who continued to live in agricultural environments but who were not further exposed to specific antigens at work, however, exhibited a recovery of CD4+ cells with a decrease in CD8+ cells and an increase in the CD4:CD8 ratio to normal levels after six months.

Further work on the nature of the CD8+ lymphocyte populations involved in the alveolitis found in symptomatic antigen exposed patients has demonstrated that both a higher proportion and absolute number of these cells express activation markers. These include the p75 chain of the interleukin-2 (IL-2) receptor, Very Late Activation antigen (VLA-1) and HLA-DR antigen [22]. The VLA-1 monoclonal antibody defines one of the molecules belonging to the integrin family and it is possible that this structure could also be involved as an adhesion protein in cell-cell interactions.

Cytotoxic mechanisms are thought to be central to the pathogenesis of hypersensitivity pneumonitis. A statistically significant increase in the number of cells with cytotoxic phenotype (including NK cells, specific and nonspecific cytotoxic T-lymphocytes) have been observed in BAL fluid of patients with hypersensitivity pneumonitis compared with controls [22, 23]. These include increased numbers of CD57+ (HNK-1) and CD56+ (NKH-1) cells and the number of such cells

also co-expressing T-cell markers predominates over the number of cells lacking these determinants. The surface membrane of these BAL cells, however, appear to lack other markers which strictly define NK cells [9].

Further evidence for cytotoxicity has been found by evaluating the relative proportions of BAL T-cells expressing the γ/Δ or α/β chains of the T-cell receptor. It has been proposed that γ/Δ + T-cells display non-major histocompatibility complex (MHC) restricted cytotoxicity and increased proportions and absolute numbers of these cells have been found in BAL fluid from patients with hypersensitivity pneumonitis [24]. Thus, the predominant lymphocyte subset found in the lungs of patients with hypersensitivity pneumonitis appears to be CD3+ CD8+ CD57+ CD56+ CD16-non-MHC restricted cytotoxic T-lymphocytes.

With regard to functional activity both cytotoxic and suppressor *in vitro* function has been demonstrated [9, 25, 26]. A significant increase in spontaneous cytotoxicity has been observed in patients with hypersensitivity pneumonitis [25], whereas BAL lymphocytes from exposed but asymptomatic farmers display *in vitro* cytotoxicity similar to that of controls [9]. Lung T-cells from patients with hypersensitivity pneumonitis have also been found to display *in vitro* suppressor activity [9, 26].

In a recent study by YAMASAKI *et al.* [27] T-cells recovered from the lower respiratory tract of patients with hypersensitivity pneumonitis were found to have decreased proliferative responses to mitogens when compared with peripheral blood lymphocytes from the same patients. This was not found to be due to the dominance of CD8+ T-cells in BAL fluid, or to the suppressive effect or impaired accessory function of alveolar macrophages. In the same study, BAL T-lymphocytes were found to express a high level of CD18 antigen (LFA-1 common β chain). This antigen is one of the surface markers strongly expressed on "memory" T-cells compared with "naive" T-cells. Furthermore, "memory" T-cells show reduced responses to mitogens when compared with "naive" T-cells [28]. Most BAL T-cells in hypersensitivity pneumonitis appeared to be "memory" cells while the relative proportions of "naive" and "memory" T-cells in peripheral blood were roughly equal [27].

It is thought that the presence of different T-cell subsets is important in regulating the appearance of granulomas and in their continued maintenance, possibly by the release of lymphokines. Furthermore, it has been demonstrated that helper T-cells are associated with active granuloma formation, whereas suppressor/cytotoxic T-cells and NK cells are associated with regression of this phenomenon [29]. Granuloma formation is not such a prominent phenomenon in hypersensitivity pneumonitis, unlike sarcoidosis where the accumulation of CD4+ cells is dominant [30]. Thus, the suppressor/cytotoxic population of T-cells found in the lungs of patients with hypersensitivity pneumonitis may be responsible for inhibiting granuloma development. Hypersensitivity pneumonitis may initially be an appropriate if exaggerated response to antigen exposure,

but if local immunoregulation breaks down, this response may become uncontrolled leading to granuloma formation and fibrosis.

In summary, activated suppressor/cytotoxic CD8+ T-cells are the predominant lymphocytes found in the lungs of patients with symptomatic hypersensitivity pneumonitis. These cells also bear some NK cell markers and markers of "memory" cells. With regard to functional activity, both cytotoxic and suppressor function can be demonstrated *in vitro* and the balance of these different functional cell types may be important in preventing progression to diffuse interstitial fibrosis.

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