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EDITORIAL

Bias toward use of T-cell receptor variable regions in the lung: research tool or clinically useful technique?

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In this issue of the Journal, WAHLSTRÖM *et al*. [1] describe the preferential use of discrete Vβ regions of the T-cell Receptor (TCR) in 12 patients with extrinsic allergic alveolitis (EAA), studied both at the time of diagnosis and after clinical recovery. In two independent manuscripts [2, 3], similar findings were demonstrated in EAA patients. These papers deserve a few comments related to: 1) the preferential use of specific V regions of TCR in normal and various pathological conditions; this type of analysis is now easier than in the past due to the availability of a series of monoclonal antibodies (MoAbs), which can detect a wide pattern of Vβ gene products; and 2) the actual significance of the bronchoalveolar lavage (BAL) pattern in the diagnosis of EAA.

The preferential use of specific V regions in normal and in various pathological conditions

Provided that a series of accessory signals are correctly delivered, T-lymphocytes recognize antigens through a receptor structure defined as TCR, strictly associated with the CD3 complex on the cell surface membrane. TCRs are heterodimers comprising either α/β or γ/δ chains, each encoded, just like immunoglobulins on B-cells, by rearranged variable (V), diversity (D), joining (J), and constant (C) gene segments during T cell ontogeny [4]. Use of MoAbs for the α, β, γ, and δ chains of the TCR that have specificity for defined V regions, together with appropriate deoxyribonucleic acid (DNA) molecular analysis of the α/β or the γ/δ TCR genes, can verify whether the cell population being dealt with is composed of: cells consistently possessing an identical TCR rearrangement ("monoclonal expansion"); cells belonging to a limited number of clones ("oligoclonal expansion"); or a multitude of cells that are different from each other ("polyclonal expansion"). The first condition (monoclonality) is detectable in well-established tumours, in which all the cells invariably originate from a single cell progenitor. The oligoclonal pattern may be consequent to the accumulation of cells from an ordered immune response that derives from antigenic stimulation, usually by a conventional peptide and as a consequence of an exaggerated, consistent antigenic pressure. Finally, the polyclonal pattern reflects either the normal condition of immunocompetent cells or the effect of stimulation by superantigens, which bind the receptor system outside the conventional antigen-binding site [5].

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On technical grounds [6, 7], the analysis of the TCR is made possible by the use of MoAbs against different Vα and Vβ regions; for the time being, the reagents that are available identify only a limited number of families. Using polymerase chain reaction (PCR) analysis, nearly the complete pattern of expression of the V region repertoire is covered; a limitation of this method lies in the difficulty in reaching a precise quantification of the individual TCR V gene. Additional techniques useful in investigating the rearrangements of TCR genes are Southern blot and random sequencing with the evaluation of the variation in length of the third complementarity-determining region (CDR3) of the TCR β-chain, which is thought to carry the fine specificity of antigen recognition by T-cells. These methods can provide the final proof of clonal disease, as seen in malignancies [8]. With respect to the pattern which is of interest in this editorial, *e.g*. the oligoclonal response, it can be polyclonal in nature, but contain expanded clones; alternatively, an oligoclonal response can be strictly oligoclonal, comprising a discrete number of specific T-cells. The distinction between these two situations may depend on the sensitivity of detection of minor *versus* major peaks in the analysis of the TCR repertoire, on the size of the sample, as well as on the sequence of the CDR3.

The central message to take home is that clonality must not be regarded as an absolute criterion of malignancy, and must obviously be interpreted in the proper context [9]. In fact, clonal populations, as shown by molecular biological analysis, have been detected during autoimmune processes, including multiple sclerosis [10], rheumatoid arthritis [11], primary biliary cirrhosis [12], and allograft rejection [13], to quote but a few. Clonal populations in these settings are likely to represent the epiphenomenon of an immunoregulatory disorder. More intriguing is the demonstration of expanded clones in healthy individuals [11, 14–17], that increase in prevalence with age [11]. This finding may denote: 1) a condition of premalignancy; 2) the result of repeated antigenic stimulation; or 3) the simple expression of an ontogenetic normal ageing process. Furthermore, studies in twins and families suggest that lymphocyte expansions in normal subjects, notably of the CD8 subtype, might arise in response to an environmental exposure [11, 15]. This is likely to be the mechanism that occurs in the lung of patients with EAA.

According to the two possibilities mentioned above, expanding lymphocytes in the lower respiratory tract of EAA patients are oligoclonal expansions over a polyclonal background (L. Trentin *et al*., unpublished). The

accumulation of cells with limited specificities may represent the consequence of a migration process of a homogeneous cell subset from the blood to this site of involvement and/or the *in situ* proliferation of antigen-specific T-cells, as a result of an antigenic pressure by foreign antigens. Since no depletion of lymphocyte subsets has been detected in the blood of EAA patients [1, 3], even in cases with a very high number of cells expressing a peculiar Vβ product in the lung, a T-cell redistribution from the blood to the lung is unlikely. The bias toward use of specific Vβ regions might then be better viewed as the consequence of the triggering of lung T-cells by a limited number of antigens, which can be found in different environments [18]. Specific determinants of *Faeni rectivirgula* might, of course, be involved in determining this T-cell selection. The *in situ* release of cytokines occurring during the development of alveolitis in these patients might represent another, though not mutually exclusive, alternative explanation of the selective expansion of particular V gene expressing subsets [19]. Other hypotheses to interpret this phenomenon (superantigens, heat shock proteins, *etc*.) are discussed in the paper by WAHLSTRÖM *et al*. [1].

Follow-up studies performed in EAA patients [1, 3] showed a close correlation between the presence in the lung of discrete populations of lymphocytes with a limited repertoire and the resolution of the alveolitis with normalization of T-cell subsets. This observation strengthens the role of putative causative antigens in determining and maintaining the lymphocytic alveolitis in EAA.

Of course, it is always central to set the degree of clonality and to distinguish the clonal dominance seen in discrete monoclonal expansions from the oligoclonal TCR-Vβ pattern identified in otherwise normal subjects [11, 14–17]. In particular, clones identified in healthy individuals appear to be relatively minor subpopulations detected by analysing the sequence of the CDR3, as compared to the clonal dominance of patients with malignancy, easily identified by Southern blot as a predominant VDJCβ rearrangement. In this context, resting BAL cells from a patient with EAA showed an oligoclonal pattern both of β and γ TCR genes, as detected by Southern blot [20].

Other than in EAA [1–3], the preferential use of discrete T-cell subsets has been identified in sarcoidosis, especially in terms of Vα2.3 expression [3, 21–23], in patients with lung involvement during human immunodeficiency virus (HIV) infection [24], and in asthma following exposure to allergen [25]. Furthermore, the common use of some Vβ regions (Vβ2, Vβ5, and Vβ6) in some of these conditions indicates that the same variable region may be used in response to different antigens. Differences in TCR-Vβ region usage detected between controls and patients suffering from interstitial lung disease suggest that the bias in using defined V regions in patients with these interstitial lung disorders is by no means related to homing mechanisms. In this context, it is worth mentioning that a diversity of the T-cell repertoire from the blood and the lung has been detected in healthy control subjects, notably the oligoclonal dominance of a few V gene families (Vα21 and Vβ9) both in CD4+ and CD8+ lung T-cells [26]. It is conceivable that this pattern reflects an environmental exposure in the lower respiratory tract, as reported in other areas, such as the skin. At this site, a preferential homing of Vβ1+, Vβ7+, Vβ14+ and Vβ16+ lymphocytes has been reported, possibly shaped by the interaction with self antigens and/or the normal microbial flora in the microenvironment of the skin [27].

The significance of the BAL pattern in the diagnosis of EAA

In the past decade, there has been considerable interest in using the phenotypic profile of BAL constituents [7], with the ultimate goal of distinguishing diseases characterized by CD4 alveolitis (*i.e*. sarcoidosis, tuberculosis, berylliosis, *etc*.) and interstitial lung disorders with CD8 alveolitis (*i.e*. EAA, lung involvement during HIV infection, *etc*.). This distinction, however, is more complex than was initially thought. For instance, it has been demonstrated that a CD8 alveolitis may occur in patients with sarcoidosis both at the onset and during the relapsing phases of the disease, the overall incidence of this phenomenon as the presenting manifestation being 3.8% [28].

As far as EAA is concerned, the most frequent pattern observed in the lung is a CD8 alveolitis [29]. In the paper by WAHLSTRÖM *et al*. [1], evidence is reported which indicates that EAA can sometimes be characterized by an accumulation of CD4+ cells. This is actually quite an unexpected finding, conflicting with the current literature on this issue [29, 30]. Given the recognition of a relevant role of CD8+ cells in the alveolitis of EAA patients, WAHLSTRÖM *et al*. [1] interpreted the pattern found in their series of cases in view of the fact that they were dealing with acute forms of the EAA, during which CD8+ lymphocytes are intensively cleared from the lung. Different phases of the disease are, thus, likely to be associated with different characteristics of the alveolitis; this has long been documented by longitudinal studies [31].

Although the incidence of the phenomenon is not reported, the possibility that a CD4 alveolitis might be sustained by a hypersensitivity process should always be taken into account by clinicians in the management of patients with allergic features. In other words, it is emphasized that the immunological analysis of BAL cell constituents may contribute to a presumptive diagnosis by enhancing or decreasing the probability of a certain diagnosis in the context of other clinical findings, but definitive conclusions cannot be drawn on the basis of the BAL profile alone.

Conclusions and outlook

The body of evidence presently available substantiates the conclusion that the analysis of the discrete usage of specific V regions in different pathological lung conditions is useful both as a research tool and for clinical purposes, obviously in the appropriate clinical context.

The possibility that *Faeni rectivirgula* determinants induce the selective expansion of a discrete subset expressing a particular Vβ region *in vitro* represents a suitable approach for investigation to definitively support the concept that an antigenic pressure determines the expansion of cells accounting for alveolitis in EAA patients.

Compelling evidence suggests that public responses (*i.e*. reproducible from individual to individual, as opposed

to private responses), exist in humans who share one or more human histocompatibility leucocyte antigen (HLA) alleles [32, 33]. The identification of these public, HLA-restricted T-cell clones associated with particular diseases, and the possibility of making this identification using a rapid technique, might of course be of help, not only for assessing the database as a current medical tool but also for designing immune manipulations. Efforts should then be pursued to determine whether or not a TCR-focused attack might downmodulate the activity of a definite TCR-type positive T-cell subset in the lung. This could set the stage for the success of a TCR-specific immunointervention in diseases characterized by abnormal expressions of TCR products. Up to the present time, this has been hampered by the unpredictable variability of the neverending specificities of the T-cell receptor.

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