

EDITORIAL

Cloning T cells from sites of allergic inflammation

Anyone who has ever dabbled in T-cell cloning will recognize the technical merits of the paper by Calder *et al.* in this month's issue of *Clinical and Experimental Allergy* [1]. T-cell cloning techniques were first developed in the late 1970s and were initially applied to cell populations that had been activated by stimulation *in vitro* with various mitogens [2]. These techniques relied upon the use of polyclonal mitogens and appropriate T-cell growth factors now known to be IL-2. Soon it became clear that under appropriate conditions, every human peripheral blood T cell could be cloned [3]. This universal clonogenic potential was demonstrated by limiting dilution analysis, which has proved a useful tool for analysing the cellular immune response in man [4]. Limiting dilution makes it possible to assess precursor frequency within cell populations and has been used to show that individuals with skin test reactivity to house dust mite have about four times as many T cells recognizing house dust mite as those who are asymptomatic [5]. When T cells are expanded by polyclonal activation prior to limiting dilution analysis, it is no longer possible to assess the precursor frequency but useful information can nevertheless be obtained concerning antigenic specificity and activation requirements in different compartments [6].

In the early 1990s evidence started to appear supporting the concept that functionally distinct T-cell subsets existed in man, just as had been previously demonstrated in the mouse [7]. Firstly, differential cytokine secretion was shown in panels of house dust mite-specific T-cell clones that were generated from two patients with mite allergy, one of whom had severe atopic dermatitis and the other of whom had allergic asthma. All the house dust mite-specific T-cell clones from the atopic donor produced IL-4 but not IFN γ , whereas the house dust mite-specific clones from the non-atopic control subject all produced IFN γ but minimal quantities of IL-4. In all three donors, clones which recognized tetanus toxoid or *Candida albicans* showed a TH1-pattern with production of IFN γ but little or no IL-4. The IL-4-producing house dust mite-specific clones supported IgE production *in vitro*, whereas the house dust mite-specific clones from the nonatopic donor did not [8]. While these clones fell into a broad TH1–TH2 spectrum, the distinction between the two poles of the spectrum was much less than that seen in murine experiments [9]. Shortly afterwards Maggi *et al.* reported increased proportions of TH2-like cells in the conjunctiva of patients with vernal conjunctivitis [10], demonstrating the importance

of studying the site of allergic inflammation rather than relying on a remote compartment such as peripheral blood.

Around the same time, several groups attempted to derive T-cell clones from asthmatic airways. The methods used to clone airways T cells varied between each group and this has hampered our ability to interpret and compare the studies. Hol *et al.* obtained BAL cells 6h after allergen inhalation, sorted T cells by flow cytometry and then cloned the T cells directly by limiting dilution with a polyclonal mitogen (PHA) but no exogenous IL-2. The cloning efficiency (the proportion of T cells that were capable of growing) was substantially less than that seen with peripheral blood T cells and the cloning conditions generated both CD4 and CD8 clones. A wide variety of cytokine phenotypes was seen amongst the various clones [11]. Pène *et al.* cloned T cells from bronchial biopsies rather than BAL. However, they cultured the bronchial biopsies in the presence of relevant allergens IL-2, IL-4 and irradiated feeder cells for 20 days before they performed a limiting dilution analysis [12]. Following limiting dilution, the T cells were stimulated with IL-2 and polyclonal mitogen (PHA). This cloning method was more efficient (cloning efficiency 11–39% in three subjects) but of course the composition of the T-cell population had been altered by the initial culture period. A total of 120 clones was obtained which varied in their cytokine profile but in the majority of the clones IFN γ predominated over IL-4. Maestrelli *et al.* cultured bronchial biopsies obtained from two patients with occupational asthma due to toluene diisocyanate. These patients had undergone allergen provocation prior to bronchoscopy. The biopsies were subjected to an initial bulk culture with IL-2 but no mitogen or antigen, prior to the limiting dilution analysis. In both cases, over 80% of clones were CD8. All the CD8 clones produced IFN γ with 43% of the clones also producing IL-5 but only 12% producing any detectable IL-4. Three months after cessation of exposure to isocyanates, no T-cell clones could be obtained from the same patients using the same methodology [13]. In our own studies, we performed BAL before and after segmental allergen challenge and then cloned T cells by limiting dilution without an initial bulk culture. This method had a very low cloning efficiency (averaging 2%) but panels of T-cell clones were obtained which demonstrated basal production of the cytokines IFN γ , TNF α , IL-13 and GM-CSF. Twenty-four hours after local allergen challenge, the clonable T cells showed a shift in cytokine repertoire towards a TH2-like pattern with less mRNA for TNF α or

IFN γ and increased expression of IL-3, IL-4 and IL-5. There was wide variation in the amount of IL-4 produced by the cloned T cells [14].

As will be clear from the publication details of these studies and the lack of subsequent work in this area, it was a difficult area to work in and it was difficult to get the information published. This is largely because of concerns that have arisen regarding the possible influence of culture conditions on the eventual phenotype of the T-cell clone. Firstly, T cells that are not capable of proliferating will not be included in the eventual analysis. This becomes an increasing problem as the cloning efficiency drops. Second, particular T-cell subtypes may proliferate better in the presence of certain cytokines. Thus TH2 cells grow better in the presence of IL-4 and IL-10 while the growth of TH1-type cells is enhanced by the presence of IL-2, IL-12 and IFN γ [15]. Moreover, single, pluripotential T cells may be biased towards the TH1 or TH2 phenotype by culture conditions. In this context, IL-4 encourages differentiation towards TH2 and TGF β seems important in differentiation towards a TH1 pattern [16]. Development towards the TH2 phenotype can also be encouraged by ligation of CD28 in the presence of IL-2 [17]. Similarly, the proportion of IL-10-producing cells is very sensitive to the IL-2/IL-4 balance in the culture medium [18]. The fact that functional heterogeneity can still be observed in T-cell clones after 6–8 weeks' culture is encouraging but does not prove that the phenotype seen *in vitro* after cloning is necessarily the same as the phenotype of the cell at the time it was obtained from the subject. Thirdly, there is evidence that only a modest proportion of T cells in blood and in the lung are specific to allergens such as house dust mite [4].

Finally, T-cell cloning has always been most appropriate to analyse those questions which require a clonal population of T cells. These include estimations of precursor frequency, detailed analysis of antigen specificity and T-cell recognition requirements, and analysis of activation and functional inactivation by allergen or by incomplete peptide ligands [19,20]. For other purposes, T-cell cloning has largely been superseded by other techniques such as intracellular cytokine staining [21,22], ELISA spot techniques and for some questions transgenic and knockout models may be more appropriate.

Against this background, Calder *et al.* [1] have provided some very useful information on the functional phenotype of conjunctival T cells in chronic allergic eye disease. T cells were isolated from conjunctival biopsies and non-specifically expanded into T-cell lines. After four to six cycles of stimulation, mixed CD4 and CD8 populations were obtained and analysed. Marked differences were observed in the T-cell lines derived from patients with atopic keratoconjunctivitis as opposed to vernal keratoconjunctivitis. The atopic group showed selective increases in IFN γ , IL-10 and

IL-13, whereas the vernal group showed IL-5 protein as well as IL-4 mRNA. This paper is useful in demonstrating a practical intermediate technology solution to the problems associated with T-cell cloning. Some problems are evident, for example the mixed CD4/CD8 populations raise some questions of interpretation since the two cell types may interact and lead to a different type of cytokine expression than would be seen from the two groups of cells grown independently. Of course *in vivo*, both cell types are present, although the degree to which they interact with each other depends on the spatial geography of the inflamed tissue as well as the relative stimulation requirements of the two cell types. When faced with such a paper, it is easy to make dry criticism of the methodology and applicability of the results. While such criticism is valid up to a point, this should not constitute a barrier to publication. The information yielded from such work is important and deserves to see the light of day. Working in human systems, especially the eyes, will always present ethical and practical difficulties and experimental studies are bound to be flawed in some respect or other. The appropriate policy for reviewers is to put the data forward and allow scientists and clinicians to use their judgement in interpreting the studies and in placing them in the context of other work.

References

- 1 Calder VL, Golly G, Hingorani M *et al.* Cytokine production and mRNA expression by conjunctival T cell lines in chronic allergic eye disease. *Clin Exp Allergy*, 1999; 29:1214–22.
- 2 Fathman CG, Frelinger JG. T-lymphocyte clones. *Ann Rev Immunol*, 1983; 1:633–55.
- 3 Moretta A, Pantaleo G, Moretta L, Cerottini J-C, Mingari MC. Direct demonstration of the clonogenic potential of every human peripheral blood T cell. *J Exp Med*, 1983; 157:743–54.
- 4 Sharrock CEM, Kaminski E, Man S. Limiting dilution analysis of human T cells: a useful clinical tool. *Immunol Today*, 1990; 11:281–6.
- 5 Khirwadkar K, Schmitz M, Kabelitz D. Frequency estimates of allergen-reactive T-lymphocytes in individuals allergic to house dust mite *Dermatophagoides pteronyssinus*. *Int Arch Allergy Appl Immunol*, 1992; 98:6–12.
- 6 Faith A, Schellenberg DM, Rees ADM, Mitchell DM. Antigenic specificity and subset analysis of T cells isolated from the bronchoalveolar lavage and pleural effusion of patients with lung disease. *Clin Exp Immunol*, 1992; 87:272–8.
- 7 Mosmann TR, Coffman RL. TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. *Ann Rev Immunol*, 1989; 7:145–73.
- 8 Wierenga EA, Snoek M, De Groot C *et al.* Evidence for compartmentalization of functional subsets of CD4 T lymphocytes in atopic patients. *J Immunol*, 1990; 144:4651–6.
- 9 Wierenga EA, Snoek M, Jansen HM, Bos JD, van Lier RAW, Kapsenberg ML. Human atopy-specific types 1 and 2 T helper cell clones. *J Immunol*, 1991; 147:2942–9.

- 10 Maggi E, Biswas P, DelPrete G et al. Accumulation of Th2-like helper cells in the conjunctiva of patients with vernal conjunctivitis. *J Immunol*, 1991; 146:1169–74.
- 11 Hol BEA, Krouwels FH, Bruinier B et al. Cloning of T-lymphocytes from bronchoalveolar lavage fluid. *Am J Respir Cell Mol Biol*, 1993; 7:523–30.
- 12 Pène J, Lagier B, Rivier A, Chanez P, Vendrell J-P, Bousquet J. Phenotype of T-cell clones obtained from bronchial biopsies and peripheral blood from three asthmatics. *Cell Biol Int*, 1993; 17:353–7.
- 13 Maestrelli P, Del Prete GF, de Carli M et al. Activated CD8 T lymphocytes producing interferon-gamma and interleukin-5 in bronchial mucosa of subjects sensitised to toluene diisocyanate. *Scand J Work Env Health*, 1994; 20:376–81.
- 14 Frew AJ. T cells in allergy and asthma. *Rev Esp Alergol Immunol Clin*, 1998; 13:241–8.
- 15 Trinchieri G. Interleukin-12 and its role in the generation of TH1 cells. *Immunol Today*, 1993; 14:335–8.
- 16 Sad S, Mosmann TR. Single IL-2-secreting precursor CD4 T-cell can develop into either Th1 or Th2 cytokine secreting phenotype. *J Immunol*, 1994; 153:3514–22.
- 17 Brinkmann V, Kinzel B, Kristofic C. TcR-stimulated naive human CD445RO T cells develop into effector cells that secrete IL-13, IL-5 and IFN γ , but not IL-4, and help efficient IgE production by B-cells. *J Immunol*, 1995; 154:3078–87.
- 18 Cohen SBA, Webb LMC, Feldmann M. The method of deriving human T-cell clones alters the proportion of IL-10-producing cells. *Immunology*, 1996; 87:343–7.
- 19 Gurka G, Ohman J, Rosenwasser LJ. Allergen-specific human T-cell clones: derivation, specificity and activation requirements. *J Allergy Clin Immunol*, 1989; 83:945–54.
- 20 O'Hehir RE, Aguilar BA, Schmidt TJ, Gollnick SO, Lamb JR. Functional inactivation of Dermatophagoides spp (house dust mite) reactive human T-cell clones. *Clin Exp Allergy*, 1991; 21:209–15.
- 21 Krug N, Madden J, Redington AE et al. T-cell cytokine profile evaluated at the single cell level in BAL and blood in allergic asthma. *Am J Respir Cell Mol Biol*, 1996; 14:319–26.
- 22 Schauer U, Jung T, Krug N, Frew AJ. Measurement of intracellular cytokines. *Immunol Today*, 1996; 17:305–6.

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