Functional subsets of lymphocytes

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In this review I shall survey various properties of lymphocytes which allow clear distinctions to be drawn between different lymphocyte subsets. I shall discuss only thymus-derived (T) lymphocytes since these cells appear to play a key role in regulating the activities of other lymphoid cells (B cells and macrophages) as well as carrying out effector functions themselves. In the first section I shall discuss murine T lymphocytes since most of the most decisive experimental studies have been carried out in this species, while the concluding section of the review will deal with human T lymphocyte heterogeneity.

Fig. 1 Differentiation of T cells.

T Lymphocyte heterogeneity in the mouse

The most useful tool for separation of T cells into different subsets has been the use of antisera to cell surface differentiation markers. Cells bearing a particular antigen may be killed by antiserum and complement or they may be detected and separated by such techniques as indirect immunofluorescence. Although T lymphocyte differentiation antigens may be detected by both heteroantisera and alloantisera (reviewed by Beverley, 1977) the most useful markers have been those detected by alloantisera. Table 1 lists some of the markers that have been particularly useful in elucidating the pathway of T cell differentiation in the mouse.

Table 1 Murine T lymphocyte surface antigens

Antigen	Linkage group	Reference
H-2	IX	Boyse and Old, 1968
Ia	IX	Frelinger <i>et al.</i> , 1974
TL	IX	Boyse and Old, 1969
Thy-1	II	Raff and Wortis, 1970
Ly-1	XII	Boyse <i>et al.</i> , 1968
Ly-2	XI	Boyse <i>et al.</i> , 1968
Ly-3	XI	Boyse <i>et al.</i> , 1971
Ala-1	?	Feeney and Hammerling, 1976

Using a panel of antisera allows the construction of a scheme for T cell differentiation such as that given in Fig. 1. This is a considerable over-simplification since many other T cell markers have been

described (Beverley, 1977; Simpson and Beverley, 1977). However, it illustrates the important point that during differentiation of T cells from stem cells resident in the bone-marrow to mature peripheral T cells there is a major reorganisation of the cell surface. Some markers (for example, T1) are lost while others appear (for example, Ala-1 which is present only on mature effector cells of both T and B lineages). In the peripheral lymphoid tissues the Ly-1, 2, and 3 markers allow a division of the T cells into three major subsets with different surface expression of the three antigens (phenotype) and distinct functional properties (Fig. 1).

Although the realisation that different subsets of lymphocytes served different functions—for example, that B cells produce antibody and T cells mediate delayed hypersensitivity—preceded the use of antisera to differentiation antigens, use of these reagents has allowed an analysis of T cell function which was previously impossible. Particularly enlightening are the studies by Cantor and Boyse (1975a, b) of mixed lymphocyte culture (MLC) responses. Their findings may be summarised as follows.

- (1) The major portion of the proliferative response in MLC is due to Ly-1⁺ cells.
- (2) The Ly-1⁺ cells respond to alloantigens coded in the I region of the major histocompatibility complex (MHC).
- (3) Cytotoxic effector cells generated in MLC and their precursors are Ly-23⁺.

Fig. 2 The mixed lymphocyte culture (MLC) responses.

(4) The target antigens of the cytotoxic cells are H-2K and D.

(5) Generation of an optimal cytotoxic response requires the presence of Ly-1⁺ cells.

These important findings are summarised diagrammatically in Fig. 2. The study established not only that different cells mediate different functions but also that the different cells respond to antigens coded in different regions of the MHC. In addition the importance of cell-cell interactions in generation of effector cells was emphasised.

Now it could be argued that the MLC is a non-physiological *in-vitro* culture artefact, and one might ask what relevance MHC recognition has to host defence against bacteria and viruses? The now classical experiments of Zinkernagel and Doherty (reviewed in Doherty *et al.*, 1976) provide an answer. They show that cytotoxic T cells, immune to viral antigens, recognise not just the viral antigens but viral antigens in association with self MHC antigens. The relevant MHC antigens are coded in the K and D regions. In addition it has now been shown that anti-viral cytotoxic cells are Ly-23⁺ (Pang *et al.*, 1976).

Does this self + antigen recognition apply only to Ly-23⁺ killer cells? Somewhat more indirect evidence than that for killer cells shows that helper cells for antibody production recognise soluble protein antigens in association with I region coded antigens (Erb *et al.*, 1976). The helper cells have also been shown to have the Ly-1⁺ phenotype (Feldmann *et al.*, 1975). In other experiments it has also been demonstrated that the Ly-1⁺ cells which mediate delayed type hypersensitivity reactions are also I region restricted (Miller *et al.*, 1976).

Taken together these data suggest that all T

Fig. 3 T cell differentiation.

lymphocytes recognise extrinsic antigen in association with self MHC antigens and, furthermore, that there are two major subtypes of T cells (Fig. 3). The Ly-1⁺ cells are helper/amplifiers for other cell types and respond to I region antigens, while Ly-123⁺ cells are suppressor/killers and K/D responsive. In addition, Fig. 3 emphasises the interactions between these two broad categories of T cell.

Human lymphocyte subsets

In this section I briefly review our present understanding of human lymphocyte heterogeneity. Fig. 4 summarises the distribution of cell phenotypes that may be found in human peripheral blood as distinguished by a number of widely used markers. These separation techniques have allowed considerable advances in understanding of human cellular immune responses but do not allow adequate definition of the non-T (non-E-rosetting) subsets nor subfractionation of the T cells.

Fig. 4 Normal lymphocyte phenotypes.

Three sets of markers have allowed a start on investigations of human T cell subsets. The first is the use of IgM and IgG rosetting. Two subpopulations of T cells can be identified by the use of ox red blood cells coated with IgM or IgG antibody. The T_M cells comprise about 55-60% and the T_G cells 10% of peripheral blood T cells (Moretta *et al.*, 1976). Complete functional studies of these subsets have not so far been reported but they show

Fig. 5 Cytotoxicity of juvenile rheumatoid arthritis (JRA) sera. Three JRA sera were assayed on nylon column purified human PBL (> 90% E+) using a two-stage ⁵¹Cr release assay with absorbed rabbit serum as a source of complement.

differences in response to PHA. More importantly, it has also been demonstrated that the T cells required to potentiate a B cell response to pokeweed mitogen reside in the T_M subset. T_G cells, on the other hand, inhibit this response (Moretta *et al.*, 1976).

There are more complete data on the use of two different types of antisera to distinguish T cell subsets. Schlossman and his colleagues have investigated the properties of sera from patients suffering from juvenile rheumatoid arthritis (JRA). Active sera have an autoantibody capable of killing up to 40% of human peripheral blood T cells (Strelkauskas *et al.*, 1978). In our experience the sera are rather more heterogeneous (Fig. 5) giving variable levels of plateau killing of peripheral blood T lymphocytes. Nevertheless, in Schlossman's hands several high killing sera have given repeatable results in studies of lymphocyte function (Strelkauskas *et al.*, 1978; Schlossman *et al.*, 1978).

An alternative approach is to develop specific heteroantisera. Rabbit antisera to T lymphocytes may be absorbed with autologous B lymphocytes (Evans *et al.*, 1977), or T leukaemia cells (Evans *et al.*, 1978) to derive sera recognising subsets of T lymphocytes. In this way two markers TH1 and TH2 have been identified. Table 2 summarises (and simplifies) the data derived from studies with JRA sera and anti TH1 and TH2 sera. It appears that most of the functions measured can be ascribed to two major subsets of lymphocytes—the TH1⁺ helper/amplifier, which appears broadly equivalent to the mouse Ly-1⁺ cell, and the TH2⁺ JRA⁺ suppressor/killer, broadly equivalent to the mouse Ly-23⁺. It is interesting that the cells which give an *in-vitro* proliferative response to soluble antigen do

Table 2 Summary of data derived from studies with juvenile rheumatoid arthritis (JRA) sera and anti-TH1 and TH2 sera

Function/response	Antigens		
	TH1 ⁺	TH2 ⁺	JRA ⁺
MLC	+	—	—
HELP	+	—	—
CML	ND	+	ND
Suppression	—	+	+
Soluble antigen	—	—	+

not fall clearly into either category. Similarly in the mouse it is unclear which subset of T cells is most important in this response. In summary, it appears that distinctions, both functional and phenotypic, between the major lymphocyte subsets have been conserved during mammalian evolution.

Since it is now clear that major subsets of human lymphocytes having distinct surface and functional properties can be defined, we might ask the relevance of these to the pathogenesis of disease. As yet data are scanty, but there is already evidence for disturbances in numbers of T_M and T_G cells in certain immunodeficiency and autoimmune disorders (Moretta *et al.*, 1977) and in multiple sclerosis (Santoli *et al.*, 1978). Preliminary evidence also suggests that JRA patients with detectable auto-antibody lack circulating suppressor cells (Schlossman *et al.*, 1978).

Rapid progress may be expected in this area when the existing markers are improved. Present hetero-sera suffer from limited availability, difficulty in reproducing successful immunisations, and multiple specificities present in the same serum. JRA sera are not readily available and only untreated patients have usable titres. Furthermore, the number of cells killed may vary (Fig. 5), suggesting multiple specificities within the sera. Perhaps the most promising avenue for advance is the use of somatic cell hybridisation to produce monoclonal antisera (Kohler and Milstein, 1976). Already an antigen present on human thymocytes but not peripheral T cells has been defined (McMichael *et al.*, 1978) which is perhaps equivalent to the mouse TL antigen. It is to be expected that peripheral T and B cell markers will shortly be described.

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