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TDR-6 **The split within the CD4 (helper) T-cell subset, and its implications for immunopathology**

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*** presenting paper**

This paper reviews progress in the splitting of CD4 (helper) subset of T-cells. This split constitutes the most exciting recent development in cellular immunology, and is closely related to an equally exciting development in molecular immunology, the molecular cloning of lymphokines.

It is a subject that came to general attention a year ago, and lead references can be found in our review at the last International Congress of Immunology[1]. Since then important new information has accrued concerning the distribution lymphokines, synthesis among T-cell subsets, the molecular biology of the CD45 membrane glycoprotein marker and the immunogenetics of MHC class II molecules in relation to immune suppression genes. Indications have begun to appear of how the split affects susceptibility and resistance to infectious disease, particularly in human leprosy and schistosomiasis, and in animal models of leishmaniasis. These developments have greatly strengthened understanding of the split and its importance: what was a hypothesis has become a guiding principle. At the same time this clarification also draws attention to the gaps in our understanding: for instance the linkage between immunogenetics and

lymphocyte function is still tenuous; in many systems immune suppression by T-cells has been inferred but not yet demonstrated directly; marker studies on CD4 T-cells need to be lined up with function; and discrepencies between man, mouse, and rat need to be re-examined.

We have argued elsewhere[2] that the greatest current challenge is to reconcile lineage partitioning of the CD4 lymphocytes (eg in terms of restriction element) with partitioning in terms of labile markers such as CD45R versus CD45R'. If the two do line up, as seems likely, we shall need to think afresh about the immunoregulatory system in terms of activation compartments that particular lineages enter preferentially. One senses that we have reached the limits of reductionism.

Why the CD4 split matters

The importance of the split is summarised in Table 1, which requires little comment as most of the points have already been made[1]. That the split may determine immediate versus delayed-type hypersensitivity is new, and emerges from in vitro studies which show that IL-4 (a product from one side of the split) upregulates both IgE synthesis and mast cell proliferation in what seems to be a coordinated way. This contrasts with IL-2 and γ-IFN (products of the other side of the split) that mediate DTH.

TABLE 1 The importance of the CD4 split

The balance of activity between the two types of CD4 cell may determine:

(i) The outcome of the immune response, in activating T versus B effector cells (ie cellular versus humoral immunity).

(ii) the magnitude of the immune response, through control of the balance between helper and suppressor T-cells.

(iii) resistance versus susceptibility particularly to chronic infection, by parasites and intracellular bacteria.

(iv) the level of immunopathological damage in chronic infection, and probably also in auto-immune disease

(v) the nature of the hypersensitivity that generates immunopathology, particularly mast cell/IgE-mediated immediate type versus T-cell-mediated delayed type.

(vi) evolutionary specialization of MHC class II genes, so that some become more polymorphic and exercise predominantly Ir (positive, immune response) function, while others become less so and exercise predominantly Is (negative, immune suppresson) function.

The nature of the split

Fig 1 summarises the nature of the split. This figure is slightly revised from our previous review, to take account of new evidence concerning CD45(T200) markers and to rectify a previous mistake in the assignment of one of them (Ox-22), to emphasise the importance of chronic infection in selecting for Is genes, and to include the new data about the pattern of lymphokine synthesis. The point of this figure is to show how CD4 T-cells divide up into two subsets when examined in at least four different ways: by markers, by their functional interactions with effector cells and their patterns of lymphokine secretion (these two may be ultimately the same), by their restriction, and by connectivity. It very much remains to be resolved whether all these splits overlap exactly. Probably they do not, if only because as has been mentioned some of these characteristics are labile while others are lineage-fixed. Nevertheless there is certainly a great deal of overlap, that represents a wide-ranging synthesis.

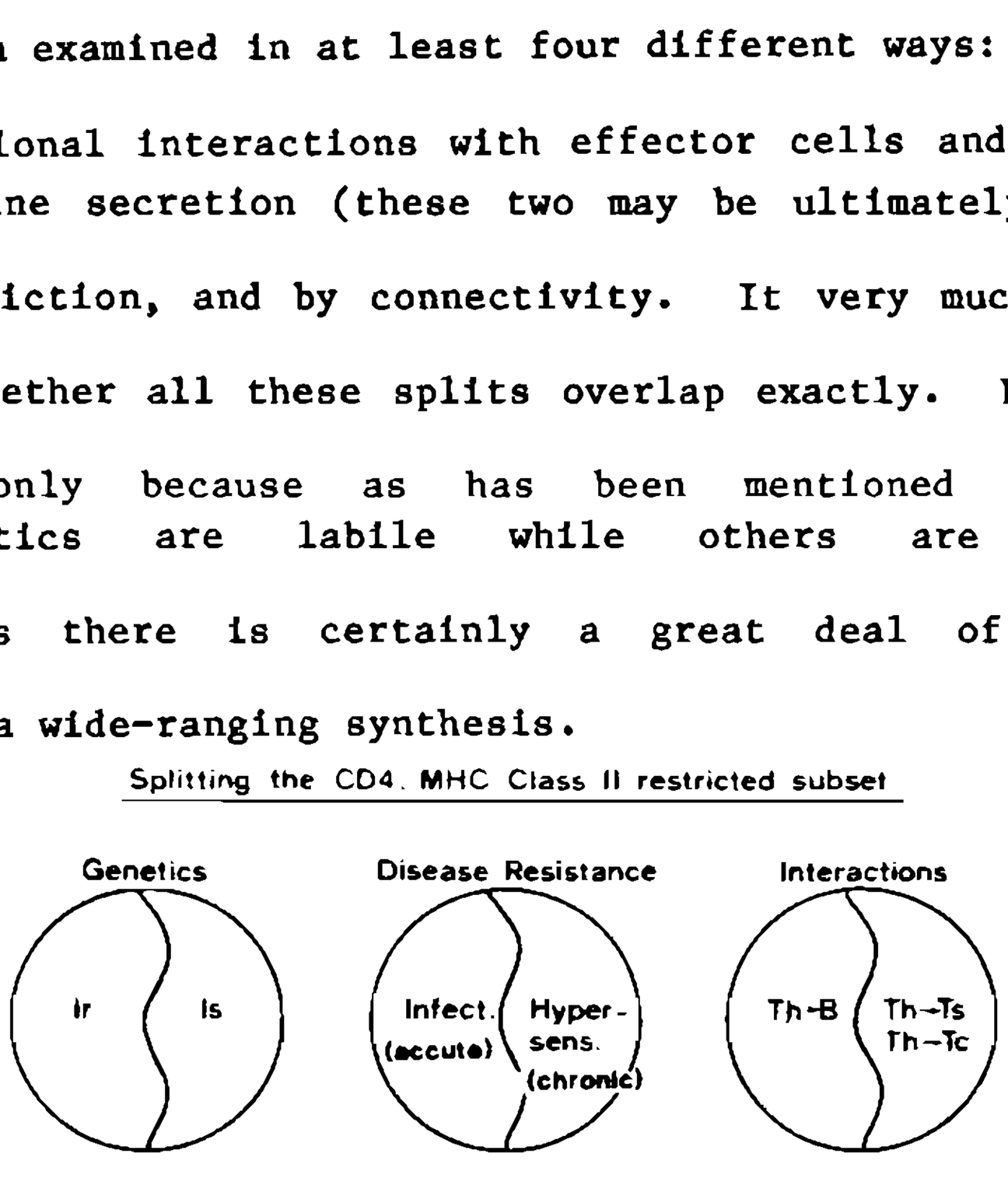

Fig 1 The split among CD4 (Class II-restricted) helper T cells. The circles represent the different properties of these

cells, and the division within each circle represents the split. Note that the divisions may not all overlap completely, so that the circles can be thought of as slightly rotated with respect to one another.

Rather than argue about priority in detail, we note that this figure covers important contributions from J Klein and T Sasasuki in the MHC class II immunogenetics respectively of mouse and man, of S Schlossman and P Beverley in the T200 markers of man and of D Mason in rat, of J Trowbridge and N Barclay in T200 molecular biology and of T Mossman and D Mason with the lymphokines.

New evidence: lymphokines

Table 2 lists the lymphokines (cytokines synthesised by T-cells) that have been molecularly cloned, and gives for most of them their assignment to the Th1 and Th2 subsets[3]. IL-6 at the time of writing has been cloned but not yet assigned. The assignment was made on the basis (a) of functional assays of the lymphokines, usually as growth factors for various cell lines, and (b) of dot blots for mRNA. Thus far these assays have been carried out only on panels of T-cells grown as clones, so that the phenotypes and frequencies in non-activated cells remains unknown. Via dot blots, or perhaps via in situ hybridization, we shall probably soon learn about the exact relationship between lymphokine synthesis pattern and surface markers.

TABLE 2

MOSSMAN'S PARTITION		
	Th1	Th2
IL-2	+	-
IFNγ	+	-
IL-4	-	+
IL-3	+	+
IL-5	-	+
GM-CSF	+	+

The lymphokine synthesis patterns clearly distinguish between T-cells that regulate B- but not T-cells via IL-4 and IL-5, and others that regulate effector T-cells via IL-2. But the division is not complete, as B-cells can respond to high concentrations of IL-2, and there may be other overlaps.

Surface markers

A year ago the surface markers were in confusion, as a whole variety of monoclonal antibodies had been found to split the CD4 set in various more-or-less equivalent ways that make no obvious sense. Most of these antibodies now turn out to belong to the CD45 cluster and to recognise epitopes on related T200 proteins encoded by alternatively spliced transcripts. This has greatly simplified matters, as many human markers now fall in line, and homologous markers can be identified in rat and mouse. Nevertheless it should be remembered that a residue of other widely used but poorly understood CD4-splitting monoclonals fall outside this cluster, including 4B4 and Leu-8 in man and Qa-1 in mouse.

Present understanding of CD45 is set out in Figs 2, 3 and 4. As shown in Fig 2, the sub-terminal, distal domain of T200 undergoes alternative splicing that generates alternative sets of epitopes named CD45R and CD45R', so called because each of these sets occur only a restricted group of lymphocytes. Proximal to this is a constant domain bearing CD45 epitopes that mark all leucocytes. CD45R monoclonals recognise the higher molecular weight proteins, and are easier to raise than those of CD45R' type, presumably because they recognise sequences that are absent from the lowest molecular weight protein. In fact only one CD45R' monoclonal has been raised, against the human form of T200[2]; whereas monoclonals

for both rat and mouse CD45R homologues are available[1,2].

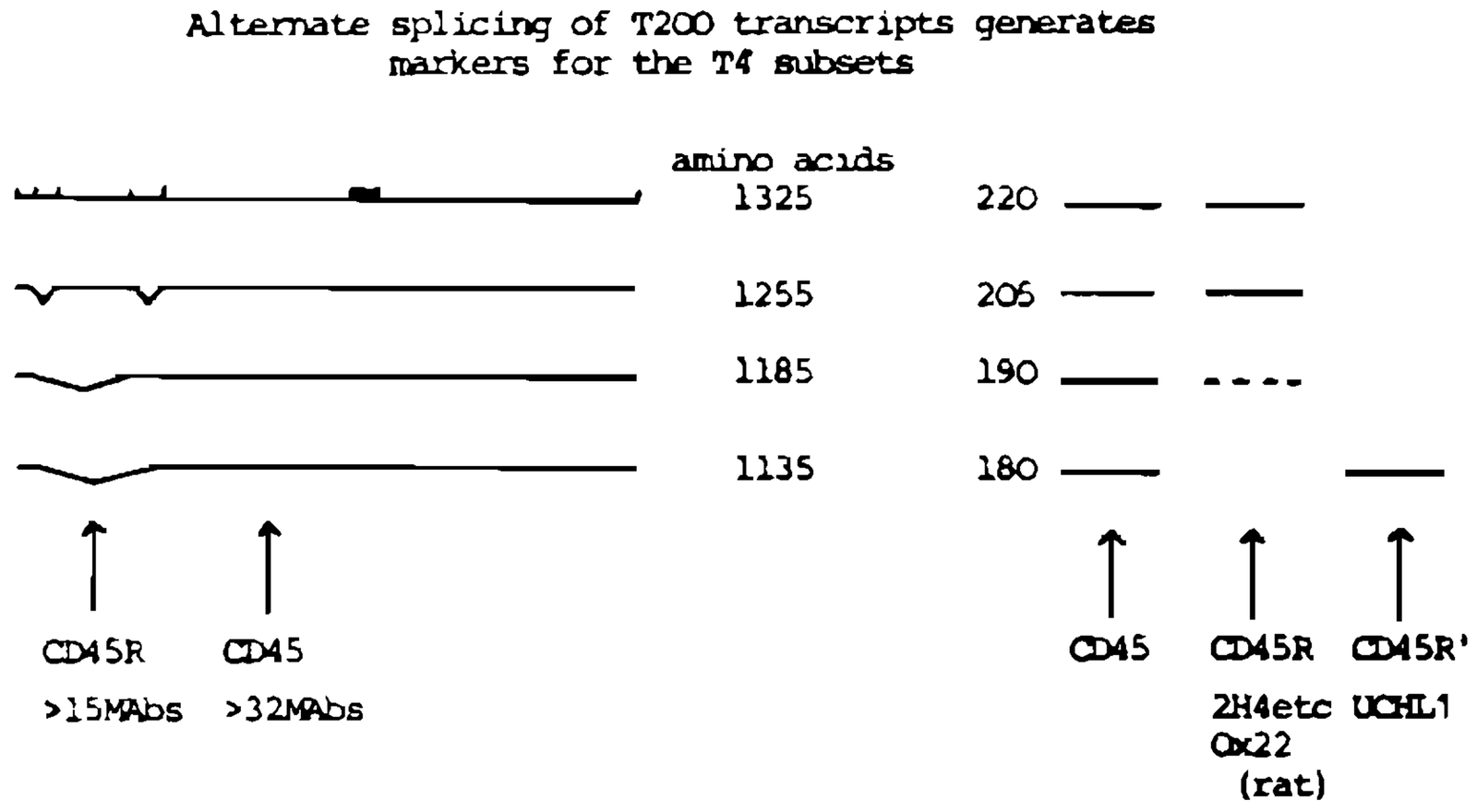

Fig 2 The molecular biology of T200 alternative splicing, from [4].

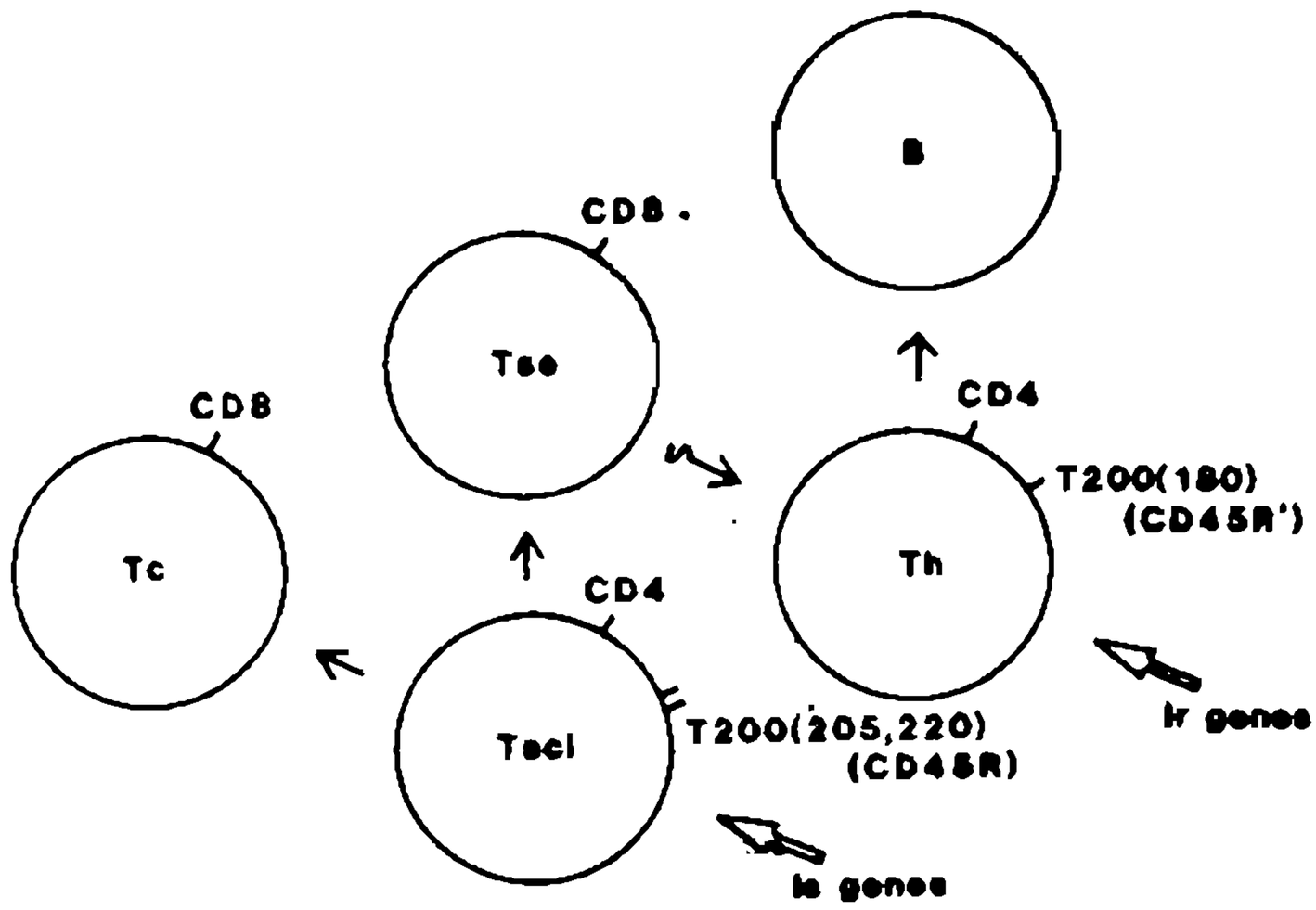

Fig 3 Functional relationships between effector cells (Tc, cytolytic T cells, and B cells) and regulatory T cells (CD45R Tsc1, suppressor/cytolytic cell inducers, CD45R' helpers for B cells and Tse, suppressor-effector cells).

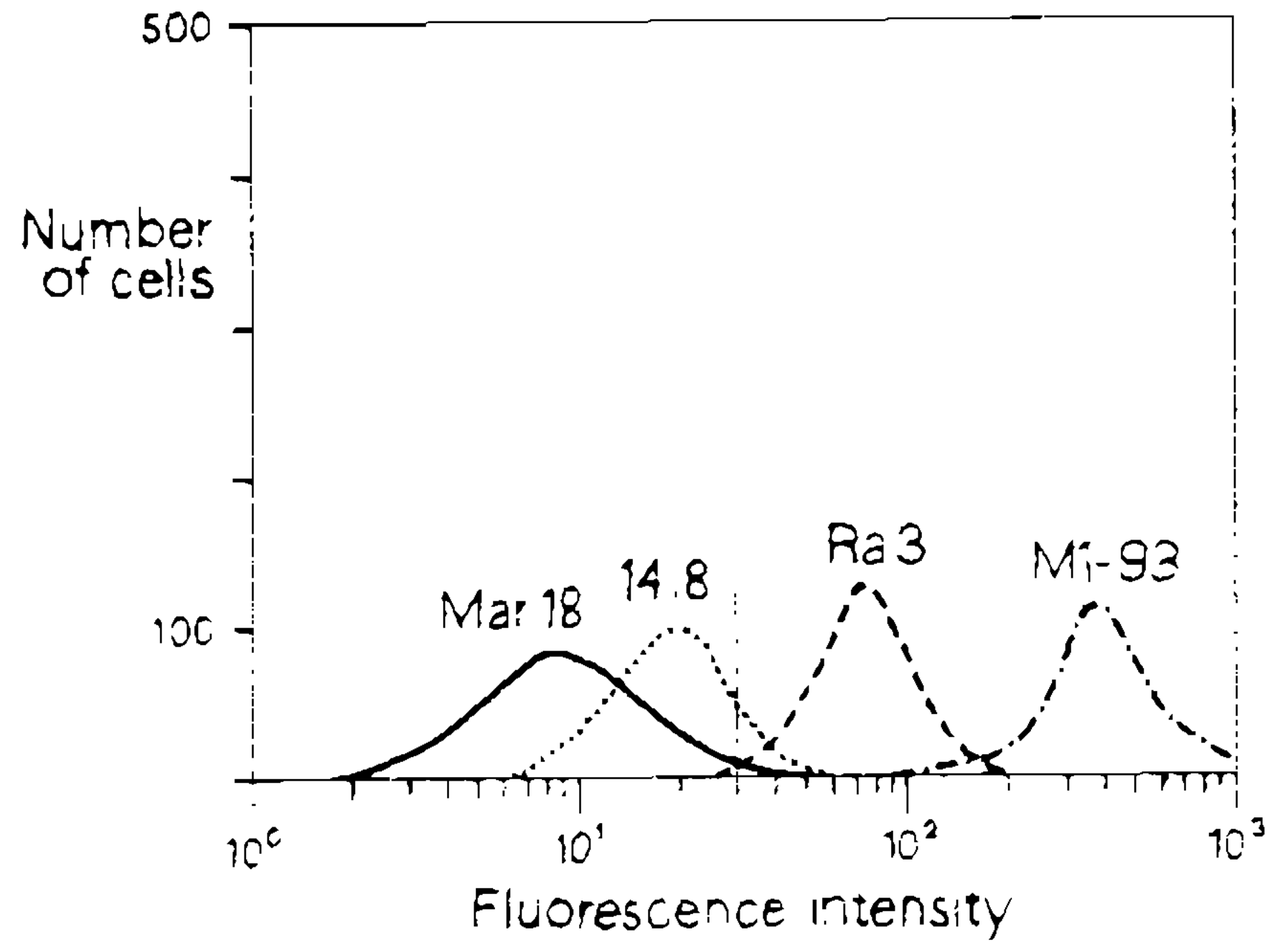

Fig 4 Staining of a T suppressor clone (Ts4.2, gift of N Nanda) of CBA anti-B10.BR specificity with various rat anti mouse T200 monoclonal antibodies[2]. 14.8 is specific for the heavy molecular weight form of T200 (220-240kD). RA3.3A1/6.1 is specific for the same molecular weight T200 but recognises another epitope on the molecule. M1-93 4HL.2 recognises all the different molecular weight forms of T200 (180 to 240 kd). MAR18 is the second layer antibody and is a mouse monoclonal specific for Rat Ig K light chain. The cells were analysed using a FACS analyser; the negative controls correspond to cells incubated only with the second layer MAR128.

The functional relationships shown in Fig 3 have been worked out in the rat, and in man[1]. Thus far little functional analysis has been performed in the mouse, but our recent data shown in Fig 4 indicate that the same general picture holds for that species, although the frequency of CD45R, CD4 T-cells seems to be suprisingly low (∿2%)

The relationship between the CD45R and R'CD4 T-cells is controversial and may not obey any simple rule. Our own colleagues' work indicates that many but not all of the CD4 T-cells that respond to a recall protein antigen (tetanus toxoid) switch phenotype from CD45R to R', so that the latter behaves mainly as a maturation marker.

So far nearly all our information concerns T200 as a marker. Our data indicate that it can transduce signals into mouse lymphocytes, but do not determine whether this occurs physiologically.

Fig 3 above indicates of where the MHC call II molecules that

exert immune response (Ir) and immune suppression (Is) effects are believed to act. They are shown here serving as restriction elements for CD4 T-cells, and therefore as MHC class II molecules. As information about Is genes accumulates, they continue to map without exception to class II loci. Furthermore much new information confirms the rule laid down last year[1], that Is activity is not distributed at random among class II genes, but tends to focus at one specialised locus within each species. The information concerning Is gene location for the three best-studied species is summarised in Fig 5.

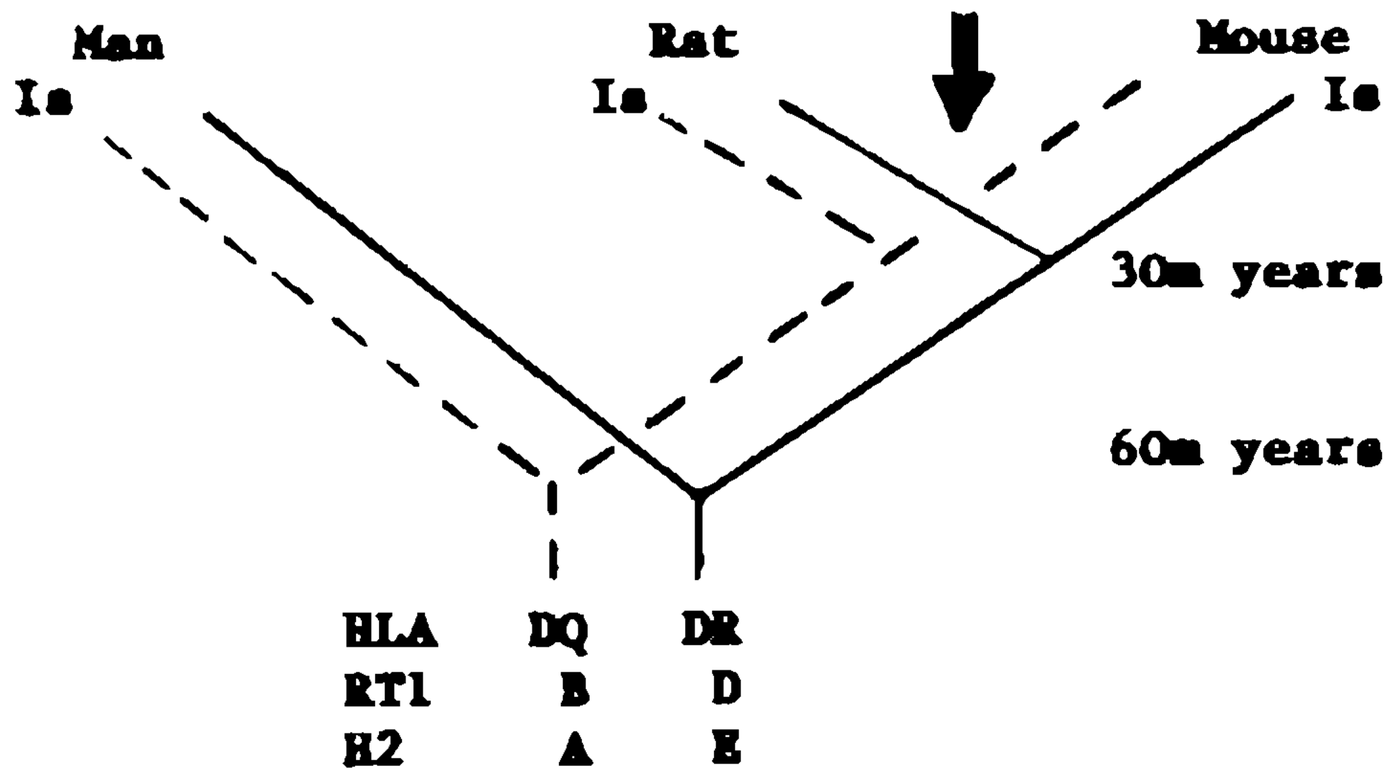

Fig 5 The evolution of MHC class II genes specialising in Is (immunosuppression) function. This figure summarises work already cited [1] plus supporting data from mouse[5-8]m man [9-12], and rat[13].

Peptide and DNA sequences establish that H-2E and HLA-DQ are not homologous, and that it is H-2E and HLA-DR that evolved from a common ancestral gene some 20 million years ago. This implies, as indicated in the figure, that Ir function is labile in evolution and appears to have flipped from the H-2A ancestral gene to H-2E in the mouse stock after the divergence from rat some 30 million years ago. Specialization is incomplete within each species, with H-2E clearly displaying some Ir function for certain antigens, so that lability of function seems to be a general rule.

Our view of how specialization evolved is shown in Figures 6 and 7. The guiding idea is that Is genes have been selected largely by chronic infection, rather than by auto-immune disease or need for homeostatic balance within the immune response. We envisage selective pressure on parasites to develop antigens able to generate immunpathology, so that the host in turn will be selected for Is gene function; this in turn will benefit the parasite as shown in Fig 6. In support of this view we would argue that fitness is reduced in the host generally not by parasitization per se, but by the consequences of immunological conflict between host and parasite. If parasite and host engage in this kind of collusion then neither will be under selective pressure to escape through polymorphism, and Is function will tend to gather at one locus so as to leave the other free to develop the high level of polymorphism that is associated with Ir function, as shown in Fig 7. This explains why it is the less polymorphic loci H-2E and HLA-DQ that exercise Is function, even though they are not homologous.

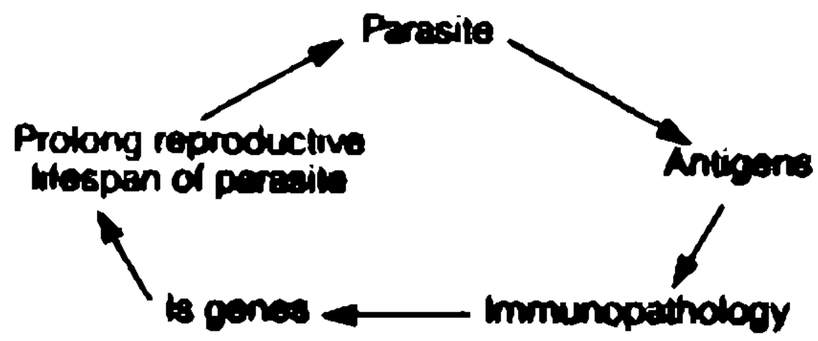

Figure 6 A feedback loop that selects for Is function during evolution, from [14]

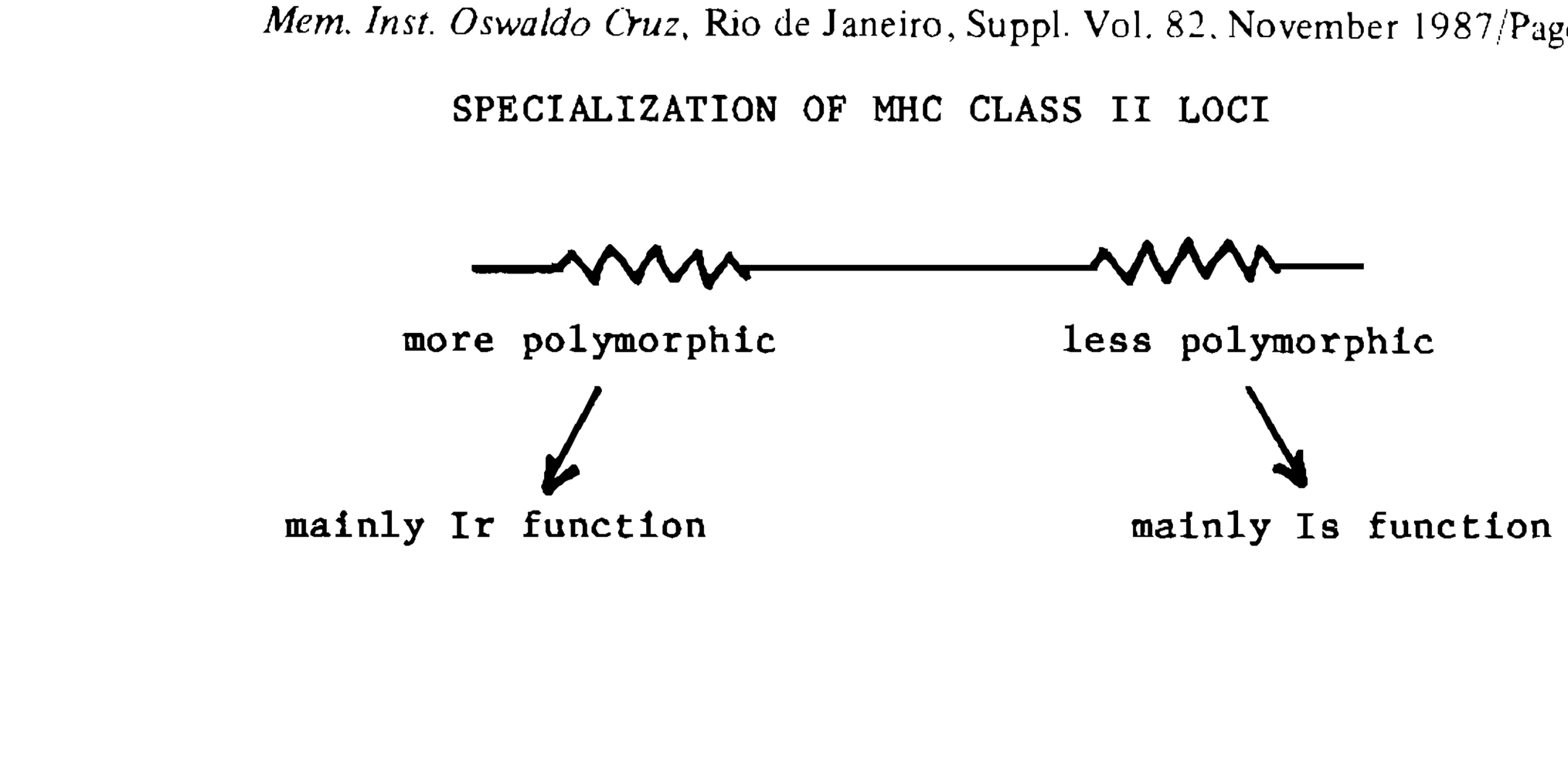

Figure 7 The evolution of Is function is likely to be associated with reduced polymorphism.

Practical opportunities

We conclude with a list of some of the possibilities in parasite disease relevant to the present symposium that are suggested by the forgoing theoretical framework.

(i) In diagnosis and monitoring, CD45R and CD45R' monoclonal antibodies can be used to determine the surface phenotype of CD4 T-cells. A high CD45R/CD45R' ratio (in tissues and perhaps also in peripheral blood) should predict low antibody levels and low immunopathology, and vice versa. The main problem here is in the expression of these epitopes on other leucocytes, particularly macrophages. Double staining is cumbersome but might overcome this problem.

(ii) In histopathology, differential expression of HLA-DQ versus HLA-DR may relate to activation of the suppressor pathway. Much further work on this topic is needed during chronic infection and the genesis of histopathology.

(iii) In treatment, improved understanding of the lymphokine synthesis profiles of lymphocyte subsets, coupled with the availability of

recombinant lymphokines, should point the way to lymphokine therapy designed to fill gaps in the immune response. For example IL-2 and γ-IFN are both likely to enter clinical trials of topical therapy in leprosy.

(iv) In much the same way one can envisage anti-lymphokine therapy. This might take the form of neutralizing monoclonal antibodies. Or it might be appropriate to administer antagonistic lymphokines; under certain conditions in vitro, for instance, γ-IFN can antagonise some of the effect of IL-4 on antibody production.

(v) Ultimately, a gap in the lymphokine patterns might be sealed by autologous adoptive transfer. Lymphocyte clones belonging to a diminished sub-set might be expanded in vitro, and then transferred back into the patient.

(vi) In terms of prevention, the principle need is to find vaccine antigens and adjuvants able to activate differentially the two pathways shown in Fig 3. This is closely related to the problem of defining helper and suppressor epitopes, that has been so much discussed recently. The rules that govern helper and cytotoxic T-epitopes are rapidly becoming clear, and we are optimistic that suppressor T-epitope analysis will make comparable progress.

(vii) A further step in the development of second generation vaccines may be to combine lymphokines with selected epitopes, perhaps by insertion of the appropriate genes into a live vector such as vaccinia virus or a wounded mycobacterium.

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