Mem. Inst. Oswaldo Cruz, Rio de Janeiro, Suppl. Vol. 82, November 1987/Page 311

Recognition of epitopes of Infectious antigens.

A. Rees, D.B. Young & J.R. Lamb

MRC Tuberculosis and Related Infections Unit

Royal Postgraduate Medical School

Hammersmith Hospital, Ducane Road

London W12 0HS

The study of the cellular mechanisms involved in the immune response to mycobacteria, essential for the design of sub-unit vaccines, requires the identification of the antigenic epitopes of molecules recognized by T cells. In man this is frequently achieved by the clonal selection of individual T cells together with a variety of methods to determine their fine specificity.

Despite the limitations of this process i.e., the possibility that T cell clones are not representative of the polyclonal repertoire and the use of purified antigens which may change the pattern of immunodominance, this is a means of evaluating which determinants may be of important biological function. For these reasons, however, it is also necessary to explore methods of determining the specificities that are immunodominant in an unselected polyclonal T cell repertoire. In this paper we shall discuss some of the methodology that has been developed in order to evaluate the fine specificity of both clonal and polyclonal T cells using either a purified epitope or a crude antigenic mixture as the initial stimulus.

1) Recombinant antigens.

Recombinant DNA antigens from Mycobacterium tuberculosis cloned and expressed in a λGT11 library of E. Coli (Young et al., 1985) can be used to identify the specific antigen recognized by T cell clones originally induced with crude antigenic mixtures. Thus a panel of T cell clones were isolated from the pleural effusion of a tuberculosis patient stimulated with M. tuberculosis soluble extract (MTSE). When these clones were initially tested with a panel of antigenic preparations from various species of mycobacteria although some were specific for only the inducing antigen the majority showed various patterns of cross reactivity (Lamb et al.,

1986). Analysis of the specificity using phage lysates from the λGT11 recombinant library showed responses that were consistent with the cross-reactivities observed in the preliminary assesment. For example, the T cell clones, P48 and P57 responded in the presence of antigen presenting cells when stimulated with the λGT11 clone expressing the 19kD antigen and also reacted to other mycobacterial species known to contain this molecule. Screening of the specificity of T cell clones in this manner permits the identification of the stimulating antigen. Further definition of specificity by the location of T cell determinants within the molecule can then be achieved by the construction of a set of recombinant DNA clones containing overlapping fragments of the structural gene of the protein. As illustrated in Fig 1 this method was used to map T cell epitopes in the 65kD antigen. Lysogens prepared from this sublibrary together with the product of the whole gene (Y3150) and the λGT11 without insert DNA as controls were used to stimulate both polyclonal (donor P ascities) and clonal human T cells (Lamb et al., 1987). The results showed that two areas within the 65kD molecule induced strong T cell responses. Interestingly, neither overlapped with B cell epitopes defined by screening a similar sublibrary with monoclonal antibodies (Mehra et al., 1986). Thus this approach can be used to generate maps of antigens showing the location of both B and T cell epitopes.

2. Predictive methods

A complementary approach to the definition of T cell epitopes involves the use of predictive theories which have been developed in order to determine which linear sequence of amino acids is likely to form a T cell epitope. As T cell responses can be obtained with linear sequences containing as few as 6 amino-acids the number of epitopes in any one molecule is potentially large and any means of limiting the

number to be studied is, therefore, of value. One such method is based on the observation that peptides which function as T cell determinants contain a distinctive amino acid pattern consisting of a charged or a glycine residue followed by two or three hydrophobic residues and terminating with a polar amino acid (Rothbard, 1986). We have tested the potential of this predictive method using a T cell clone (P77) with specificity defined by its responses to the λGT11 sublibrary prepared from the gene encoding the 65kD molecule. As can be seen in fig 1 and discussed above the use of sublibrary clones enabled the stimulating epitope to be approximately (within 50 amino acids) located. Six peptides containing the motif were identified and synthesized (Lamb et al., 1987) together with a control peptide which only contained a B cell epitope (Mehra et al., 1986). The capacity of the selected peptides to stimulate proliferation of the T cell clone P77 and a polyclonal T cell population (donor P ascites) was determined. It was found that the TLC P77 only recognized the peptide (residues 112-132) that corresponded to the same region of the 65kD molecule previously identified by the overlapping 65kD recombinant sublibrary approach (fig 1). Similar results were obtained with the polyclonal population where two regions were identified, one identical to the site recognized by the clone (viz residues 112-132) and the other within residues 437-459. Both these regions contain two potential epitopes (fig 2), although which of these is actually recognized by the T cells has not yet been determined. Interestingly, T cells reactive with the sequence 437-459 also proliferate in response to E. coli (Young et al., 1987) which is consistent with its location within a region of the 65kD molecule (residues 361-468) that is highly conserved with a related protein in E. coli and probably other bacteria.

3. Direct probing of the repertoire.

At present the obtainable antigens, both purified and recombinant, are defined by the binding of mouse monoclonal antibodies. Whilst the consistency with which the

antigens are obtained in various laboratories suggests these may be immunodominant it is probable that T cells will also respond to other mycobacterial antigens and these may also be of importance in T cell responses. Consequently it is necessary to directly probe the T cell repertoire and thus avoid potential constraints imposed by preselecting antigens with monoclonal antibodies. This can be achieved by stimulating polyclonal T cells in the presence of accessory cells with antigens bound to a solid phase matrix such as nitrocellulose (Young & Lamb, 1986). Thus when MTSE is fractionated on SDS-PAGE and immunoblotted 20 x 2mm fractions are obtained. Analysis of the response of polyclonal T cell populations to these fractions shows distinct patterns of peaks and troughs in the proliferative responses (fig 3). Whilst these responses are frequently lower than those observed with soluble antigens they nonetheless have the potential to identify recognition patterns in different populations and thus to identify molecules of importance in for example diseased as compared to normal subjects. Although the peaks obtained from peripheral T cells of normal subjects have been found to be predictable, particularly in the molecular weight range 80-90, 50-65, 24-28 and 12-18kD, the responses from T cells of tuberculous lesions can show different patterns of responses (fig 3). Although these studies may show qualitative differences in responding populations it is less easy to make quantative evaluations as peaks may reflect either the concentration of particular antigens or of T lymphocytes of appropriate specificity. It is unclear whether the "troughs" observed reflect the absence or the presence of determinants recognized by regulatory T cells. The observation that the response of purified CD4 T cells also show peaks and troughs would suggest the former is more likely to be the case unless the regulatory population also has this phenotype.

This method can also be used to determine the molecular weights of antigens within complex antigenic mixtures recognized by T cell clones (Lamb & Young,

1987). Thus testing of the responsiveness of TLC P53 and P57 indicated that the former responded to fractions corresponding to a molecule within the molecular weight range 16-18kD, whilst the latter recognized a slightly higher molecular weight antigen 18-20kD. As only TLC P57 reacted with the recombinant 19kD antigen this confirmed that the fine specificity of T cells could be analysed in this way. The observation that other TLC (e.g. P35) responded to antigens in molecular weight ranges (52-55kD) not so far defined by monoclonal antibodies indicates that there are indeed T cell epitopes that are distinct from those defined by the mouse B cell repertoire.

4.Affinity purified antigens and antibody

Antigens obtained by affinity purification have been used to stimulate the polyclonal T cell responses (Young et al., 1986) as well as to identify the specificity of M. leprae generated T cell clones (Ottenhof et al., 1986a). As previously discussed determination of fine specificity is more readily performed with recombinant antigens or peptides. Indeed as evidence by experience with the 38kD molecule the failure of the recombinant library to express a particular antigen can result in some difficulty in precisely defining T cell specificity. Despite the fact that antibody responses to this molecule provide the best discrimination between healthy individuals and tuberculosis patients T cell responses are found to an equal degree in both groups (Young et al., 1986). Furthermore, mouse model studies suggest that the response to this antigen may be under Ir gene control (Ivanyi & Sharp, 1986). More extensive population studies in man have, however, been precluded by the difficulty of obtaining sufficient quantities of purified antigen. In these circumstances, it is of interest that anti-idiotypic antibodies containing T cell stimulatory internal images can be generated. The rabbit anti-idiotypic antibody (anti-Id TB71) (Praputpittaya & Ivanyi in press) raised against the idiotype

of the monoclonal antibody TB71 (Coates et al., 1981) stimulates a T cell clone reacting with the 38kD molecule (Rees et al., 1987a) in a manner that is MHC restricted and independant of the three dimensional structure of the antibody (Rees et al., 1987b). The results are consistent with the presence within a structural domain of the antibody of a linear sequence that mimics an epitope in the 38kD molecule. Preliminary results suggest that if this is the case then the sequence is located on the light chain of the antibody. Current studies are directed towards the identification of this sequence. In this way it will be possible to obtain an antigenic probe for a molecule that has not so far been accessible. The existence of such an antibody considerably facilitates study of the T cell responses to this specificity. Furthermore, it also has important implications for strategies aimed at vaccine design.

Conclusion

Several methods of determining the fine specificity of T cells have been described. These methods do not all rely on the generation of T cell clones but rather permit a more flexible approach to an evaluation of the T cell repertoire responding to mycobacteria. The identification of T cell epitopes in mycobacterial antigens is important for considerations of vaccine design. Additionally, synthetic peptides corresponding to these epitopes can also be used to evaluate their importance in the immune response. Thus comparisons between patients and vaccinated protected individuals might indicate which specificities are associated with both responses.

The methodology discussed so far has been used to examine the fine specificity of CD4 T cells. The role and the fine antigenic specificity of the regulatory or cytotoxic CD8 T cell subpopulation are also of some importance. Murine studies

(Townsend et al.,, 1985) indicate that these cells also recognize peptides in association with MHC determinants. We have also used these methods to probe the fine antigenic specificity of CD8 T cell clones and have found that they also respond to a recombinant antigen in the λGT11 library. It has not, however, been possible, as yet, to precisely define the epitope recognized. Nonetheless, these findings do suggest the methodology described can be applied to both T cell sub-populations.

Acknowledgements

We thank Mrs E. Hall for the excellent typing of this manuscript and N. Dobson, A. Scoging, L. Kent & A. Mistry for excellent technical assistance.

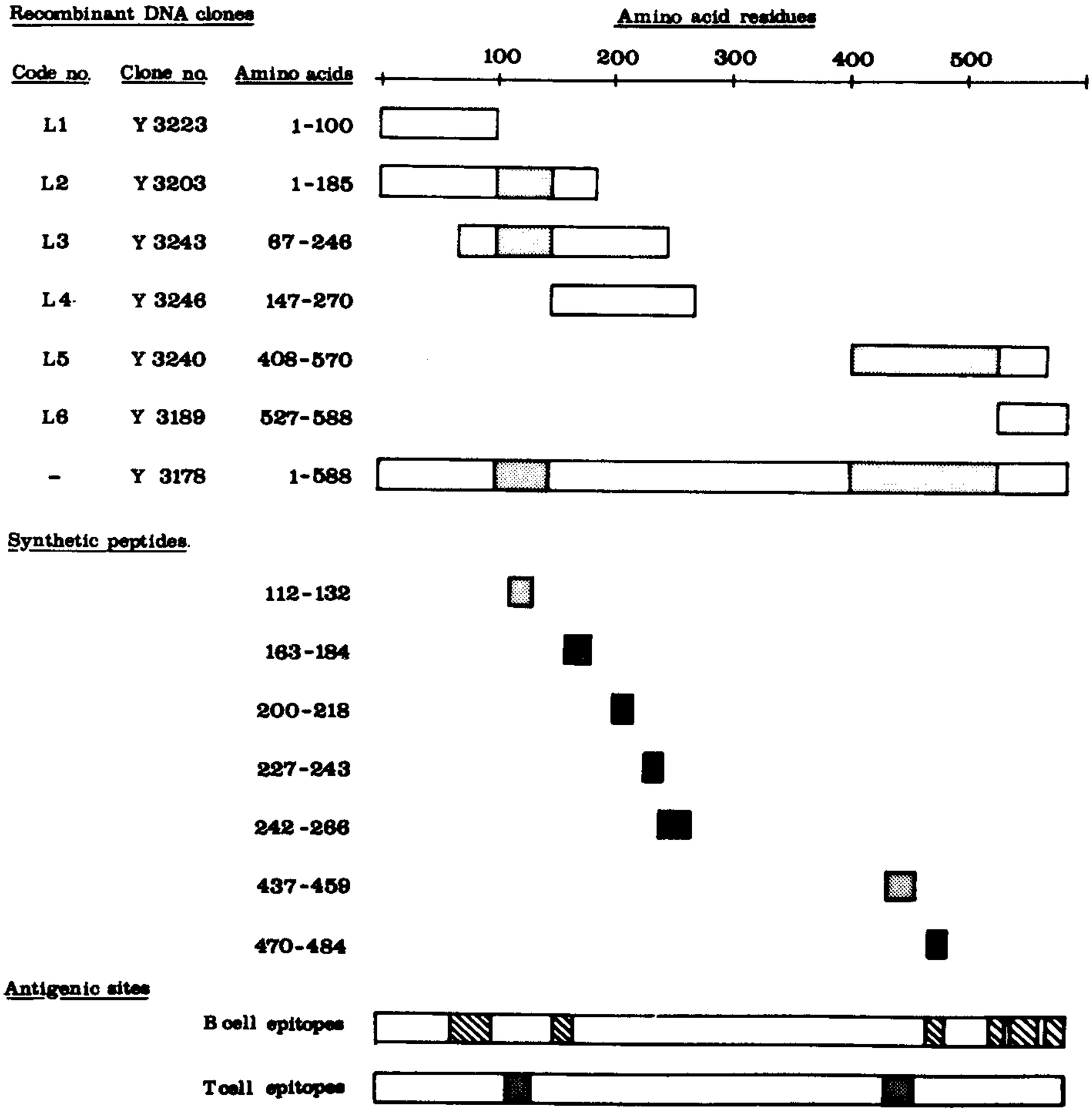

Fig.1 Map showing areas of the 65kD protein covered by DNA sublibrary clones and synthetic peptides. The amino acid residues of the 65kD protein (1,588, based on proposed M.leprae coding sequence) are shown on the scale along the top. The upper portion of the diagram shows the areas of the gene covered by the DNA sublibrary clones, while the lower portion shows the synthetic peptides. The stippled areas correspond to the regions recognized by T cells (Lamb et al., 1987).

112 132
Y E K I G A E L V K E V A K K T D D V A G

437 459
R K H R I E D A V R N A K A A V E E G I V A G

Fig. 2. Amino acid sequence of residues 112-132 and 437-459 of the 65kD protein. Amino acids are represented by the single letter code. The underlined residues represent the characteristic patterns associated with the presence of predicted T cell epitopes (Rothbard, 1986).

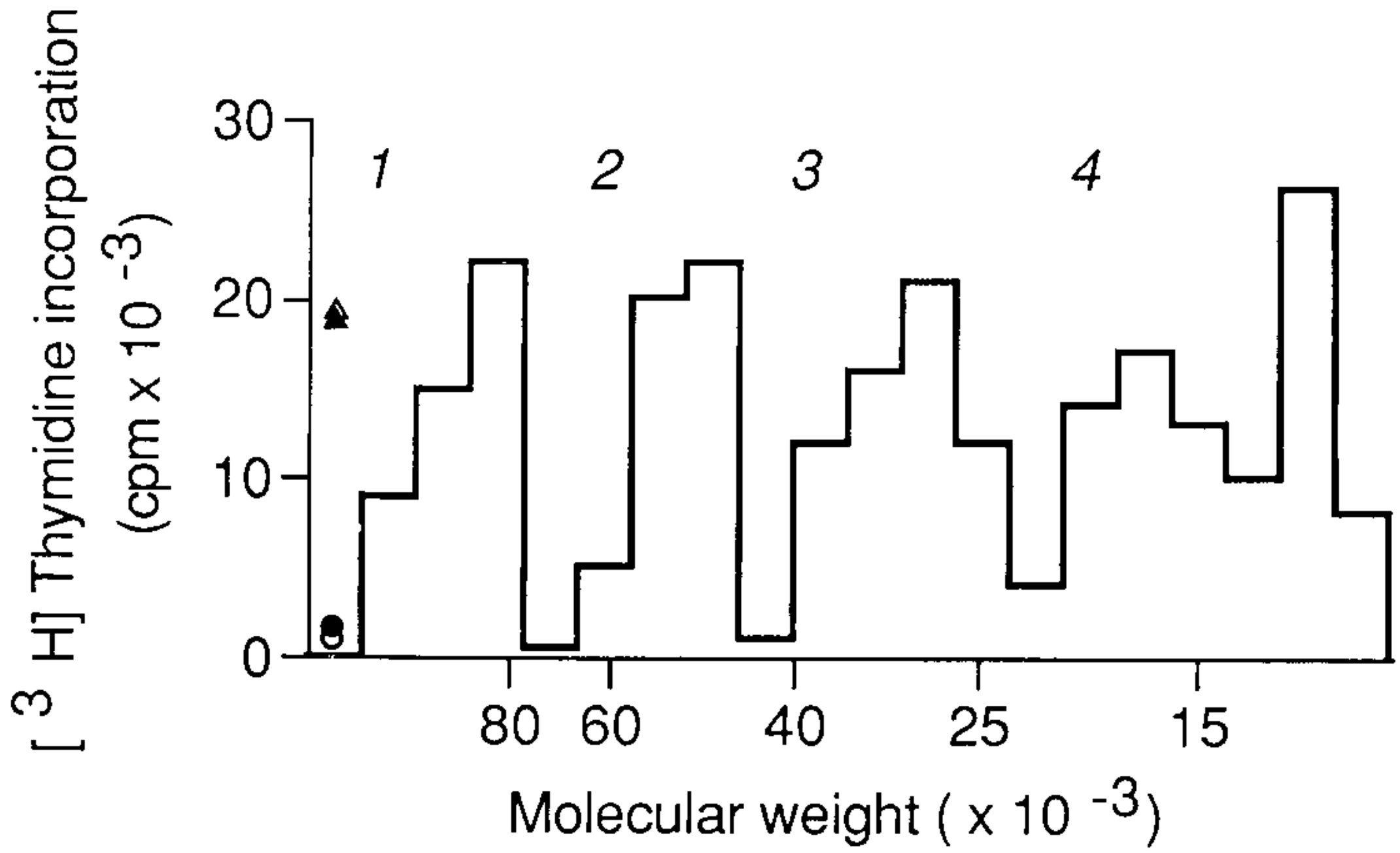

Fig. 3. Identification of M.tuberculosis antigens recognized by polyclonal T cells using immunoblot analysis. These were cultured at 10^5/ml SDS-PAGE separated immunoblots (20 fractions) together with irradiated PBMC (a) / ascitic lymphocytes (b) at $5x10^5$/ml as a source of antigen presenting cells. The results are expressed as the mean cpm ± SEM of triplicate cultures. Control responses of T cell to medium (o), APC without antigen (●) and soluble antigen ± the presence of nitrocellulose (▲) (Young & Lamb, 1986).

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This publication received financial support from the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases.