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The identification of T cell epitopes in *Mycobacterium tuberculosis* using human T lymphocyte clones

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Introduction

Strategies for the design and development of subunit vaccines to protect against infectious disease depend upon the identification and characterisation of immunodominant epitopes of important biological function and specificity present in the pathogen (1). Since effective immunity during mycobacterial infection appears to be mediated predominantly by the cellular arc of the immune response, (2,3) this necessitates the detailed analysis of the specificity of T cell recognition of mycobacterial antigens. In this report we review some of the recent advances that have been made in this area of mycobacterial research.

Selection of mycobacterial antigens for immune analysis

The generation of murine monoclonal antibodies directed towards *Mycobacterium tuberculosis* and *Mycobacterium leprae* have been the primary means by which antigens are selected for the investigation of human immune responses during infection. This has allowed a number of protein antigens to be characterised (4-6) and together with the appearance of antibodies with overlapping specificities this would suggest that certain mycobacterial determinants are immunodominant as regards the murine B cell repertoire (4,5). Recently these antibodies have been used not only for the immunochemical purification of antigens (7), but also in the screening of recombinant DNA libraries for antigen expression (8-10). Thus the antigens employed in probing the repertoire of T cell specificities in response to mycobacterial infection or immunisation have been selected by antibodies. Not only do T cells recognize protein antigens by qualitatively different mechanisms from those used by B cells (11), but also appear to react with epitopes discrete from the B cell binding sites within a molecule (12,13). Therefore although the antibody defined antigenic sites in mycobacteria may be dominant for murine B cells, it is unclear whether the hman T cell response will reveal the same pattern of reactivity.

T cell responses to mycobacterial antigens

The recognition that interleukin 2 (IL-2) can maintain human T lymphocytes in long term culture (14) has allowed the isolation of monoclonal populations of T cells that retain antigen specificity and biological function (15). Using this technology to isolate T cell clones from tuberculosis and leprosy patients cellular reagents have become available for the functional

analysis of critical T cell epitopes between different species of mycobacteria. T cell clones induced with the soluble extract of *M. tuberculosis* H37Rv (MTSE) were isolated from the ascitic effusion of a tuberculosis patient and their specificity pattern analysed on different species of mycobacteria (Table 1).

Table 1.
Specificity of M. tuberculosis *induced T cell clones for various species of mycobacteria.*

	Clone No.				
ANTIGEN	P35	P48	P53	P57	P77
M. tuberculosis, H37Rv	+++	+++	+++	+++	+++
M. bovis	–	++++	++++	++	+++
M. leprae	–	–	++	–	++
M. avium	–	–	++	–	+
M. kansasii	–	+	++	+	–
APC	–	–	–	–	–
Medium	–	–	–	–	–
IL-2	++	+	++	++++	++

Cloned T cells isolated by limiting dilution (15) were stimulated with various species of mycobacteria in the presence of irradiated histocompatible PBMC as antigen presenting cells. proliferation as correlated with ^{3}H-TdR was determined at 72 hr. –,<500 cpm; +, 1000-2000 cpm; ++, 2000-5000 cpm; +++, 5000-10000 cpm; ++++, >10,000 cpm.

Of the T cell clones assayed all with the exception of P35 showed various patterns of cross reactivity. Clone P53 was broadly cross reactive recognising a determinant(s) present in all the species tested (Table 1), and the pattern of reactivity of clone P77 was similar except that it failed to respond to *M. kansasii.* In contrast P35 appeared to be specific for only the inducing antigen MTSE. Although this clone has failed as yet to respond to any of the preparations of *M. bovis* tested, (12 in total), we are unable to conclude that this clone is specific for a unique epitope in the *M. tuberculosis* (H37Rv) until the panel of mycobacteria has been extended. Clones P48 and P57 demonstrated a different pattern of specificity in that they proliferated predominantly to *M. tuberculosis* and *M. bovis,* with reduced reactivity to *M. kansasii* (Table 1). Specificity analysis of H37Rv induced T cell clones isolated from a BCG vaccinated individual (16,17) support these findings that the major component of the T cell response to *M. tuberculosis* is cross reactive with that directed against *M. bovis.* Similarly cross reactive T cell clones from leprosy patients have been identified (18,19). However as regards the recognition of *M. leprae* derived antigens a much greater proportion of the T cell response is against species-specific epitopes as determined at the clonal level (17,18). It is of interest to note that the diversity of the murine B cell repertoire to *M. tuberculosis* is more cross reace as compared to that directed against *M. leprae* (4-6).

Recognition of mycobacterial antigens expressed in *E. coli*

The cloning and expression of the genes for the major protein antigens of *M. tuberculosis* and *M. leprae* as defined by monoclonal antibodies (8,9) now allows T cell recognition of individual molecules to be assessed which had previously been restricted by the availability of purifies antigens. Using recombinant DNA antigens it has been reported that *M. leprae* spe-

cific human T cell clones recognize predominantly epitopes within either the 18Kd (17) or 36Kd (18) proteins. However the specificity of the clones described in this latter report (18) were initially defined using antigens purified from *M. leprae* sonicate by affinity chromatography.

The same panel of clones described in Table 1 were investigated for their ability to recognize λgt11 phage lysates of *E. coli* containing *M. tuberculosis* antigens (Table 2).

Table 2.
Specificity of M. tuberculosis *induced T cell clones defined with recombinant antigens*

ANTIGEN	RESPONSE - cpm				
	P35	P48	P53	P57	P77
MTSE	*6285*	*6430*	*9767*	*5456*	*8270*
MBSE	405	16706	12897	4488	5602
3150 (65Kd)	255	441	799	254	*21269*
*4143 (65Kd)	313	340	263	315	5227
3151 (14Kd)	373	190	279	402	4184
4147 (19Kd)	325	*6142*	345	*7534*	503
*4272 (71Kd)	287	233	960	908	2193
3111 (71Kd)	250	146	322	793	572
λgt 11	344	365	447	984	731
APC	546	768	413	689	774
Medium	614	212	626	649	352
IL-2	4223	4187	4264	16808	2325

* Antigen expressed as fusion protein linked to β-galactosidase.

Lysogens of λgt11 clones containing recombinant *M. tuberculosis* DNA (8) were prepared in *E. coli* Y1089 and grown with induction at 42°C in the presence of IPTG as described previously (20). Bacterial pellets were sonicated and supernatant material was fractionated by precipitation in the presence of 50 % saturated ammonium sulphate. Ammonium sulphate precipitates were resuspended in PBS and analysed by Western blotting with appropriate monoclonal antibodies. the monoclonal antibodies used were; 65Kd, IT13 (TB78); 14Kd, IT20 (TB68); 19Kd, IT19 (TB23) and 71KD, IT11 (5IA) (4,5). The samples (10μg/ml) were added to cultures containing T cells clones and irradiated histocompatible PBMC and proliferation determined at 72hrs. Results are expressed as mean cpm of triplicate cultures, sem < 20%.

The T cell clones P48 and P57 responded in the presence of antigen presenting cells when stimulated with the λgt11 clone 4147 which expresses an antigenic determinant of the 19Kd protein (Table 2). However no proliferation was observed in response to the other recombinant antigens or the λgt11 control. The presence of this protein antigen in both *M. tuberculosis* and *M. bovis* as demonstrated using monoclonal antibodies (4-6) is consistent with the cross reactivity pattern of these T cell clones. In addition to responding strongly to the 65Kd recombinant antigen clone P77 also appeared to recognise less demonstrably a determinant(s) present in the extracts from recombinant clones expressing the 14Kd, 19Kd and 71Kd antigens. It is possible although unlikely that an invariant determinant is shared

within these molecules that is absent from *E. coli* since the λgt11 control fails to stimulate P77. Clearly futher investigation of the specificity of this clone will be of interest. Emmrich and his colleagues (21) have generated T cell clones from a leprosy patient stimulated with *M. leprae* and from normal individuals that are specific for a recombinant 64Kd protein of *M. bovis* BCG. Clones P35 and P53 were not stimulated by any of the antigens expressed by the recombinant DNA clones tested. Further advantages of the use of recombinant DNA antigen is that by manipulation of the insert size the fine specificity of the T cell clones can be established bypassing the need for the synthesis of large numbers of overlapping peptides.

Application of SDS-PAGE separated nitrocellulose bound antigen in defining T cell antigen recognition

To overcome the constraints of antibody selection of antigens for probing the cellular immune response to mycobacteria we have adopted a procedure involving T cell recognition of antigens which are fractionated by polyacrylamide gel electrophoresis (SDS-PAGE), transferred onto nitrocellulose (22) and then added directly to proliferation assays (23,24). Preliminary experiments demonstrated that unfractionated antigens blotted on nitrocellulose could be recognized by T cells (23). T cell activation in these cultures was dependent on accessory cells since nitrocellulose bound antigen alone or supernatants obtained by preincubation of membranes in medium without accessory cells failed to induce a response in proliferation assays. This suggested that the mechanism for the T cell response involves active processing (25) of the solid-phase antigen by accessory cells rather than leaching of antigen from the nitrocellulose. Following the observation that T cells can recognize solid-phase antigen, MTSE was fractionated on SDS-PAGE transfered to nitrocellulose, and added to T cell proliferation assays. This approach was used to determine the molecular withts of antigens recognized by the T cell clones induced with total cellular extract of *M. tuberculosis* (Table 3).

Despite responding to nitrocellulose-bound MTSE albeit with markedly reduced proliferation (30-50 % reduction), the molecular weight of the antigen recognized by clone P77 could not be idnetified by SDS-PAGE immunoblots (Table 3). However, the cross reactive clones P53 and P57 recognized determinants associated with the 16-18Kd and 18-20Kd molecular weight immunoblot fractions respectively. this confirms the specificity of P57 for the 19Kd protein as established by recombinant DNA antigens (Table 2). T cell clone P35 which proliferated only in response to *M. tuberculosis* extract responded to an antigen migrating in the molecular weight range of 52-55Kd (Table 3). At present no monoclonal antibody recognising this molecule has been generated. Thus it would appear that this approach of screening T cell specificity can lead to the identification of T cell antigenic sites on proteins which are not recognised as immunodominant by mouse antibodies.

Concluding remarks

While it has been possible to resolve the antigen specificity of some of the T cell clones induced with unfractionated preparations of *M. leprae* or *M. tuberculosis* by affinity purification or recombinant DNA technology, the initial selection of antigenic determinants has relied on antibody recognition.

One consequence of this is to bias the analysis of the T cell repertoire towards antigens or molecules recognised by both T and B cells and exclude those determinants that are primarily T cell antigens. In part this may be overcome by the use of SDS-PAGE fractionated nitrocellulose bound antigen as a direct probe for T cell specificity at the clonal level and for repertoire analysis of polyclonal populations. The ability to demonstrate the specificity of a T cell clone for the 19Kd protein by either analysis with recombinant antigen or immunoblots con-

Table 3.
Specificity of M. tuberculosis *induced T cell clones defined by SDS-PAGE separated nitrocellulose bound antigen (immunoblots).*

	Clone No.				
ANTIGEN	P35	P48	P53	P57	P77
[a] Soluble MTSE	++++	++++	++++	++++	++++
[b] MTSE	+++	++	++++	+++	+++
[c] MTSE imunoblot	52-55Kd	–	16-18Kd	18-20Kd	–
[d] MBSE immunoblot	–	–	16-18Kd	18-20Kd	–
nitrocellulose control	–	–	–	–	–
APC	–	–	–	–	–
Medium	–	–	–	–	–
IL-2	++++	++++	++++	++++	++++

Cloned T cells were stimulated the soluble extract of *M. tuberculosis* (MTSE) as [a] soluble antigen or [b] blotted on nitrocellulose in the presence of irradiated histocompatible PBMC. Immunoblots of (c) MTSE or (d) *M. bovis* BCG soluble extract (MBSE) were prepared as previously described. Briefly, MTSE or MBSE was separated by polyacrylamide gel electrophoresis under denaturing conditions (SDS-PAGE) and transferred to nitrocellulose. Each nitrocellulose blot was divided into sections for addition to proliferation assays. After 72 hrs. incubation the cultures were resuspended and the nitrocellulose strips discarded prior to the addition of ^{3}H-TdR.

firms the specificity and resolution of this latter technique. However it is not without its shortcomings in that not all clones recognize immunoblotted antigens and that the sensitivity of the assay system may not be adequate where the proliferative response to soluble antigen is minimal. Nevertheless this technique using T cell clones or lines depleted of *E. coli* reactivity may be of value for the screening of nitrocellulose bound gene products from recombinant DNA libraries as an approach in the identification of λgt11 clones expressing T cell antigenic determinants.

We have also used the immunoblot system for analysis of polyclonal T cell responses to mycobacterial antigens and have identified peaks of maximal response associated with antigens in particular molecular weight regions (24). A combination of such a polyclonal approach along with the techniques described here for clonal analysis would appear to offer the most effective procedure for dissection of the overall T cell repertoire. In addition, further investigation of areas of immunoblots stimulating little or no T cell proliferation may provide a means for identification of determinants capable of directly mediating non-specific inhibition or the induction of suppressor T cells.

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