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Stimulation by anti-idiotype antibody of murine T cell responses to the 38 kD antigen of *Mycobacterium tuberculosis*

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SUMMARY

Rabbit antisera raised against eight monoclonal antibodies (MoAb) binding to distinct mycobacterial antigens revealed individual anti-idiotype specificities following cross-absorption with normal mouse globulin. Only one of these antibodies (Rb71), directed towards an *M. tuberculosis*-specific epitope on the 38 kD protein antigen was able to stimulate mouse T cell responses which had 'internal image' characteristics. This was demonstrated by anti-Id induced *in vitro* proliferation of Lyt 1.2^+ T cells and the elicitation of delayed type hypersensitivity (DTH) foot pad reactions in antigen stimulated BALB/c mice. The DTH reaction was equally strong in mice sensitized with either *M. tuberculosis* or *M. bovis* soluble extracts, thus showing a greater degree of cross-reactivity than antibody binding of the corresponding TB71 MoAb. The immunizing potency of the anti-Id *in vivo* was demonstrable in three strains of mice following injection of Rb71 emulsified with incomplete Freund's adjuvant. These mice showed 38 kD antigen induced DTH-reactions as well as *in vitro* proliferation of spleen cells. The display of T cell stimulatory internal image by anti-Id may be interpreted as a consequence of binding specificity of TB71 MoAb of a T cell epitope present in the 38 kD antigen.

Keywords idiotype T lymphocytes *Mycobacterium tuberculosis*

INTRODUCTION

The presence of determinants related to immunoglobulin idiotypes (Id) on specific T cells has been reported in earlier studies which assumed that antibody V genes participate in encoding T cell receptors for antigen (Ramseier, Aguet & Lindemann, 1977; Binz & Wigzell, 1977; Rajewsky & Eichmann, 1977). It has also been argued that T cells possess a receptor with segmental homology to the V_H region of immunoglobulins (Marchalonis, 1985). Although genes linked to the Igh complex may encode constant regions of T cell receptors, the absence of V_H gene rearrangement in T cells argues against the notion that the same V_H genes that encode antibody V regions are functional in T cell receptors (Kronenberg *et al.*, 1983). However, these advances in molecular genetics have not been fully reconciled with the demonstration that antibodies towards immunoglobulin idiotypes can stimulate antigen specific T cell responses (Thomas *et al.*, 1981; Sharpe *et al.*, 1984).

The set of existing monoclonal antibodies (MoAb) with binding specificity to several mycobacterial antigens (Ivanyi, Morris & Keen, 1985) offered an opportunity to ascertain their idiotypic composition. It was of particular interest to study the anti-Id mediated modulation of T cell responses which are known to play a mandatory role in both protective and pathogenic

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interactions in the infected host (reviewed by Ivanyi, 1986). Analysis of anti-Ids relating to anti-*M. leprae* MoAb demonstrated the induction of idiotype positive but paratope negative serum 'Ab3' responses (Praputpittaya & Ivanyi, 1987). One anti-Id, however, stimulated proliferative responses of T lymphocytes from sensitized human subjects (Rees *et al.*, 1987). In this paper we demonstrate that rabbit anti-Id (Rb71) directed towards a monoclonal antibody (TB71) specific to the 38 kD protein antigen of *M. tuberculosis* can also induce delayed type hypersensitivity (DTH) and *in vitro* lymphocyte proliferation in mice.

MATERIALS AND METHODS

Antigens. Mycobacterium tuberculosis strain H37Rv was grown for 8 weeks as a surface pellicle on Sautons medium and bacilli were killed by 2·5 megarads of ^{60}Co irradiation. A soluble extract (MTSE) was prepared by disruption of bacilli using a Braun MSK cell disintegrator at 4000 rev/min output for 2 min at 5–10°C. The supernatant was cleared of debris by being centrifuged at 100,000 ***g*** for 60 min, filtered through a 0·45 μm Millipore membrane and stored in samples at −20°C. Similar extracts were prepared from *M. bovis* and from armadillo-grown *M. leprae*.

The 38 kD protein antigen of *M. tuberculosis* was purified from the culture filtrate of H37Rv using monoclonal antibody TB71-based affinity chromatography, as described recently (Young *et al.*, 1986).

Monoclonal antibodies. Hybridoma cells which had been described previously (Coates *et al.*, 1981; Ivanyi, Morris & Keen, 1985) were grown as ascites in BALB/c mice. The globulin fraction was precipitated from the ascitic fluid with 18% Na_2SO_4.

Anti-idiotype sera. Rabbits were immunized with the MoAb preparation from eight mouse monoclonal antibodies and the antisera were extensively cross-absorbed on a normal mouse globulin (NMG) affinity column, following a procedure described elsewhere (Praputpittaya & Ivanyi, 1987). Subsequently, anti-Id antibodies were purified on affinity columns coupled with the corresponding idiotype-positive MoAb coupled to CNBr-Sepharose 4B (Pharmacia, Sweden) beads using the technique recommended by the manufacturer. Rabbit anti-Id sera, previously NMG absorbed, were passed through MoAb columns at a flow rate of 1 ml per 10 min. Unbound proteins were washed out with phosphate-buffered saline (PBS) and the anti-Id was eluted with three-column volumes of 0·2 M glycine-HCl, pH 2·5. The eluted antibody was neutralized with 1 M Tris-glycine, pH 8·0, concentrated, dialysed against PBS and the protein concentration was determined using the phenol reagent (Sigma).

Paratope competition test. This was performed by the technique described in the preceding paper (Praputpittaya & Ivanyi, 1987). Briefly, serially diluted anti-Id sera were incubated on antigen (MTSE) coated plates in the presence of ^{125}I-labelled MoAb for 18 h at 4°C. Bound radioactivities were counted and on the basis of relative binding values the 50% inhibitory titres (ID_{50}) were calculated.

Immunization of mice. Inbred mice (BALB/c, CBA.Ca, C57BL/6) aged 6–10 weeks were purchased either from Olac Ltd (UK) or from the National Institute for Medical Research, (London). Mycobacterial antigens, affinity purified anti-Id or normal rabbit globulin (NRbG) were prepared as an emulsion in incomplete Freund's adjuvant (IFA) (Difco) at a volume ratio of 1/1 and injected subcutaneously. For the induction of delayed type hypersensitivity (DTH) responses, mice were given 50 mg cyclophosphamide (CY) per kg body weight, 2 days before injection of 50 μg of mycobacterial soluble extract or 5 μg of affinity purified anti-Id antibody. Pretreatment with CY is known to potentiate DTH responses in mice (Lagrange, Mackaness & Miller, 1974; Mitsuoka, Baba & Morikawa, 1976).

DTH test. Challenge antigens in 25 μl volume were injected into left hind footpads 2 weeks after sensitization. Footpad swelling was measured using a reverse spring loaded caliper (Pocotest) 24 and 48 h after elicitation and the increase in footpad thickness ($\times 10^{-2}$ mm) between the challenged and non-challenged (right) footpads were compared.

Treatment of cells with anti-T cell antisera. Spleen cells adjusted to a concentration of 1×10^8 cells/ml in RPMI medium were incubated for 30 min at 20°C with 1/500 diluted anti-Thy-1.2

Table 1. Review of anti-idiotype sera

Molecular weight of antigen	Monoclonal antibody			Rabbit anti-Id serum	
	Code	Ig class	Cross-reaction*	Code	ID_{50} titre§
38 kD	TB71	G2b	*M. bovis* (MB)†	Rb71	700
	TB72	G1	MB[b)]	Rb72	500
14 kD	TB68	G1	MB	Rb68	100
19 kD	TB23	G1	MB + *M. kansasii*	Rb23	700
65 kD	TB78	G1	MB + *M. fortuitum*	Rb78	2,700
	ML30	G1	Broad‡	Rb30	10,000
25–40 kD	ML34	M	Broad	Rb34	3,500
	ML02	G3	Broad	Rb02	600

* Beyond *M. tuberculosis* species.
† Weak binding.
‡ See Ivanyi *et al.* (1985).
§ Paratope competition test (see methods section).

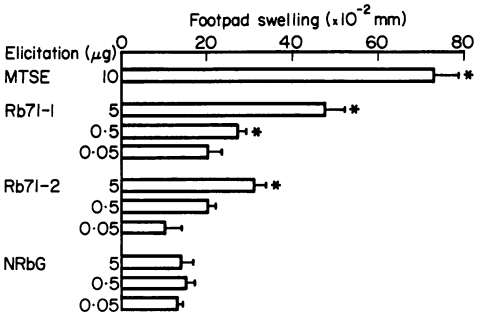

Fig. 1. Elicitation by anti-Id of DTH response in antigen-primed mice. Arithmetic means ± s.e. from BALB/c mice (six per group) pretreated with 50 mg/kg cyclophosphamide (day 2) primed with 50 μg MTSE in IFA and elicited 2 weeks later. * $P < 0{\cdot}01$. Two separate preparations of affinity purified Rb71 anti-Id were tested.

(F7D5, Olac), 1/50 anti-Lyt-1.2 (CL8912-A, Cedarlane) or 1/25 anti-Lyt-2.2 (CL8922, Cedarlane) antibodies. Cells were washed once, incubated with a 1/10 diluted rabbit complement (Low-Tox-M, Cedarlane) for 45 min at 37°C, washed twice, and viable cell concentrations were adjusted on the basis of the trypan blue exclusion test.

Lymphocyte transformation test. Spleens cells (5×10^5 in 200 μl) were dispensed into 96 well round-bottomed microculture plates (Nunc, Roskilde Denmark) with 20 μl of antigen or anti-Id in RPMI-1640 medium supplemented with 2 mM L-glutamine, 100 IU/ml penicillin/streptomycin and 10% fetal calf serum (Flow Laboratories). Cultures were incubated for 6 days at 37°C in an atmosphere of 5% CO_2 in humidified air and pulsed with 1·0 μCi of (^{3}H-TdR) (Amersham International, Amersham, Bucks) for 16 h. Cells were harvested on glass fibre filter paper, radioactive uptake was measured by liquid scintillation and expressed as the arithmetic mean ct/min ± standard deviation of the means (s.d.) from triplicate cultures.

Table 2. Specificity of DTH in BALB/c mice elicited by anti-Id Rb71

Sensitization with soluble extract	Footpad swelling* (mm × 10^{-2}) elicited by:		
	Soluble extract†	anti-Id Rb71	NRbG
M. tuberculosis	69±7*	51±3*	17±2
M. bovis	93±6*	72±5*	7±2
M. leprae	55±2*	23±4	17±2

* Arithmetic means ($n=6$)±s.e.; * $P<0{\cdot}01$ compared with saline injected controls.

† Homologous with the sensitizing species.

Table 3. Phenotype of anti-Id stimulated spleen cells

Treatment of cells	3H-TdR (ct/min±s.d.) uptake			
	MTSE (1 μg/ml)	Rb71* (20 μg/ml)	NRbG (20 μg/ml)	Saline
None	84909±10911	21442±2636	976±123	2676±186
Complement	51647±3953	21539±1083	862±64	926±116
Anti-Thy 1.2+C′	1894±200	1549±137	1200±145	1308±61
Anti-Lyt 1.2+C′	3050±443	2146±264	720±57	1594±132
Anti-Lyt 2.2+C	57216±8653	17538±4058	708±42	965±168

In vitro response of spleens from BALB/c mice immunized with 50 μg MTSE, 2 weeks before harvest.

* Mean values obtained with affinity purified preparations, derived from two separate rabbits.

RESULTS

Definition of Rb anti-Id antisera. Rabbit antisera which had been cross-absorbed with NMG were directed toward eight MoAb reacting with epitopes on five distinct antigens of *M. tuberculosis* (Table 1). The relationship of Id to the antigen binding site was determined by competitive inhibition immunoassay (PCT). It was found that all tested Rb anti-Id antisera inhibited the binding of homologous MoAb to plates coated with MTSE with a potency expressed by ID_{50} titres that varied within a range of 1/100–1/2700 range. This suggested that the anti-Id sera reacted with paratope-associated idiotypes. None of the tested anti-Id sera showed any cross-reactivity beyond the homologous MoAb (Praputpittaya, 1986).

Elicitation of DTH response in mice by Rb anti-Id. Affinity purified Rb71 anti-Id preparations from two rabbits elicited significant DTH responses in mice that had been immunized with MTSE antigen (Fig 1). Footpad swelling elicited by Rb71 anti-Id was found to be dose-dependent and significantly higher ($P<0{\cdot}01$) than that evoked by NRbG at two of the concentrations (5·0 and 0·5 μg) tested.

The specificity of DTH response elicited by Rb71 anti-Id was examined in BALB/c mice preimmunized with soluble extracts of antigens from (*M. tuberculosis, M. bovis* or *M. leprae* Table 2). The results showed that each of the homologous mycobacterial extracts elicited strong footpad swelling, in sensitized mice. By contrast, Rb71 anti-Id induced significant footpad swelling in mice

Table 4. DTH response to MTSE in anti-Id injected mice

Strain	Reaction at	Footpad swelling following priming with MTSE	Rb71	NRbG
BALB/c	24 h	84±5**	47±3**	18±3
	48 h	61±2**	33±3**	12±3
CBA/Ca	24 h	65±8**	39±2**	23±1
	48 h	46±9*	28±4	24±1
C57Bl/6	24 h	59±11**	40±5*	20±1
	48 h	42±5**	18±3	14±3

Mice (six per group) pretreated with 50 μg/kg cyclophosphamide (day-2) were primed with 50 μg MTSE or 5 μg Rb71 (or NRbG) in IFA and elicited 2 weeks later with 10 μg MTSE.

** $P<0.01$, * $P<0.05$, compared with NRbG control.

Table 5. Proliferative response to the 38 kD antigen by spleen cells from anti-Id primed BALB/c mice

Stimulant in culture medium	(μg/ml)	^{3}H-TdR (ct/min±s.d.) uptake of cells primed *in vivo* with: Rb71 anti-Id	MTSE
None		35549±490	3665±626
MTSE	1·0	5663±1108	11101±538
38 kD antigen	1·0	18318±1251	15752±1871
	0·1	13016±1206	6378±602
	0·01	7008±1452	3788±362
Rb71 anti-Id	10·0	15430±3264	10965±624
	1·0	16749±1000	37841±390
	0·1	16803±957	6504±653
NRbG	10·0	13205±2048	856±72

Spleen cells from BALB/c mice injected 2 weeks before harvest with either 5 μg Rb71 anti-Id or 50 μg MTSE in IFA.

immunized with *M. tuberculosis* or *M. bovis*, but not in mice immunized with *M. leprae*. Mice immunized with any of the three mycobacterial antigens and challenged with NRbG did not show significant reactions to the challenge material with an average mean of footpad swelling not exceeding $17\pm2\times10^{-2}$ mm.

Proliferative effect of Rb71 anti-id on MTSE-immune spleen cells. Spleen cells from two mice immunized with MTSE in IFA were stimulated *in vitro* with MTSE, affinity purified Rb71 anti-Id from two rabbits (Rb71-1 and Rb71-2) or with another two Rb anti-Id antibodies (Rb72 and Rb78) of distinct specificity (Figure 2). Rb71-1 anti-Id at 20·0 and 2·0 μg/ml concentration stimulated a dose-dependent ^{3}H-TdR uptake in MTSE-immune spleen cells from two mice. The Rb71-2

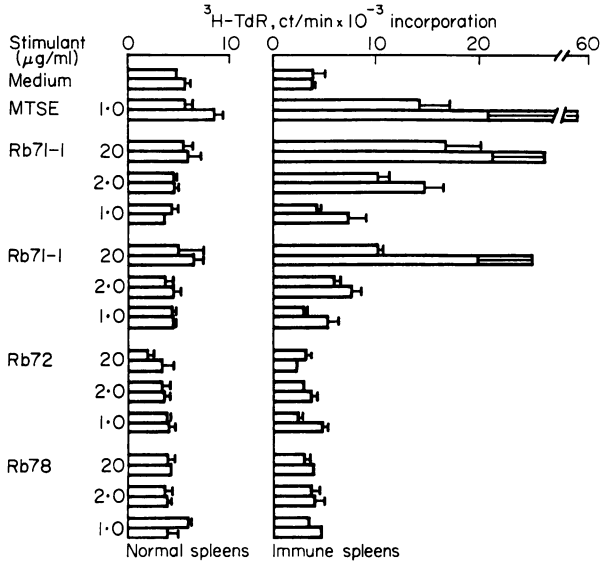

Fig. 2. Proliferative effect of anti-Id on spleen cells from antigen-primed mice. Columns represent the *in vitro* response of individual spleen cells from two mice immunized by subcutaneous injection of 50 μg MTSE/IFA and two normal mice. Cells were incubated for 6 days at 37°C in the presence of affinity purified rabbit anti-Id antibodies (Rb71-1 and -2 were derived from two rabbits) and mean values ± s.e. of ^{3}H-TdR uptake from triplicate wells are expressed.

preparation was of lower potency whereas Rb72 and Rb78 anti-Id antibodies, were not stimulatory. All tested preparations, including MTSE, failed to stimulate the spleen cells from non-immune BALB/c mice. The failure of Rb71 anti-Id to stimulate non-immune spleen cells suggests that contamination of the affinity purified Rb71 preparation with mitogenic substances such as endotoxin can be ruled out. Subsequent experiments with MTSE immune spleen cells, whilst responding to MTSE and Rb71, failed to show significant proliferative responses to anti-Id sera Rb23, Rb68 and Rb34 (results not shown).

The phenotype of cells responding to MTSE or Rb71 anti-Id, was determined by treating spleen cells from MTSE-immunized BALB/c mice with antisera specific for mouse T cell subsets (Table 3). The proliferative response of MTSE-immune cells to both the MTSE and Rb71 anti-Id was abrogated by pretreatment of the cells with either anti-Thy 1.2 or anti-Lyt1.2 plus complement. However, pretreatment of cells with anti-Lyt 2.2 had no effect or slightly increased the proliferative response. These results demonstrated that spleen cells responding to both MTSE and Rb71 anti-Id were of the Thy1$^+$, Lyt1$^+$ 2$^-$ phenotype.

Immunogenicity of anti-Id in vivo. DTH and proliferative responses to the 38 kD antigen were examined in mice sensitized with Rb71 anti-Id. BALB/c, CBA/Ca and C57BL/6 mice were injected with Rb71 anti-Id, NRbG or MTSE antigen in IFA, challenged into their footpads with MTSE in saline 2 weeks later and footpad swelling was measured at 24 and 48 h (Table 4). A significant sensitizing effect of Rb71 anti-Id as well as MTSE was demonstrable in mice of all three tested strains, with the highest responses in BALB/c mice. Injection of NRbG did not induce a significant footpad reaction to MTSE in any of the three strains.

The specificity of sensitization by Rb71 anti-Id was examined by the *in vitro* spleen cell responses to affinity purified 38 kD antigen (Table 5). The results showed that the proliferative response to 0·1–1·0 μg/ml of the 38 kD antigen of Rb71 anti-Id immunized spleen cells (13,000–18,210 ct/min) was similar or even stronger than that obtained with cells from MTSE-primed mice (6,380–15,750 ct/min). Stimulation with 1·0–10·0 μg/ml Rb71 anti-Id of MTSE primed spleen cells (37,840–10,960 ct/min) confirmed the results shown in Fig. 2. However, the stimulation of Rb71 primed spleen cells

with either Rb71 or NRbG *in vitro* was of similar extent, indicating a response to common antigens contained in rabbit immunoglobulins.

DISCUSSION

The study of T cell responses to *M. tuberculosis* is important for the identification of protective antigens involved in vaccination against the infection. Anti-Id antibodies are relevant for this purpose since they were shown to stimulate protective immunity in animals against bacterial, virus or parasite infections (Sacks, Esser & Sher, 1982; Sharpe *et al.*, 1984; McNamara, Ward & Kohler, 1984; Kennedy *et al.*, 1986). It is of particular interest that idiotypes of overlapping specificity between xenogenic or monoclonal anti-Id antibodies, and reovirus-immune T cells were sufficiently common even in heterogeneous populations of B and T cells (Ertl *et al.*, 1982).

In this paper it has been demonstrated that Rb71 anti-Id antibody stimulated *in vitro* the response of T cells from mice sensitized to the corresponding 38 kD protein antigen of *M. tuberculosis*. Whilst Rb71 anti-Id induced the proliferation of Ly 1^+ 2^- T cells, anti-ids Rb72 relating to another epitope on the same molecule (Young *et al.*, 1986) or Rb78, Rb23 and Rb68 relating to the 65 kD, 19 kD and 14 kD antigens (Engers *et al.*, 1986), did not have any T cell stimulatory activity. Since injection with MTSE sensitized mice to all these antigens, it appears that an internal image is expressed in a unique way in Rb71 but not in the other tested anti-Id antibodies. It is of interest that screening with TB71 and TB72 antibodies of an *M. tuberculosis* recombinant DNA library expressing several antigens, has so far failed to detect the 38 kD antigen (Young *et al.*, 1985). Hence, in this case the anti-Id antibody with internal image may potentially be a valid substitute for antigen.

Injection of Rb71 anti-Id into footpads of MTSE immunized mice elicited an antigen-specific footpad swelling response. This reaction was dose dependent and exhibited the characteristics of a delayed-type hypersensitivity response with a peak at 24 h and a decline afterward (Collins & Mackaness, 1968). Specificity experiments on the DTH response showed that only mice immunized with soluble extracts from *M. tuberculosis* and *M. bovis*, but not from *M. leprae*, showed DTH footpad responses to challenge with Rb71. Although the TB71 antibody only weakly cross-reacts with its B cell epitope on *M. bovis* (10% when compared with *M. tuberculosis*), cross-reactivity for the recognition of the 38 kD antigen by T cells is apparently more pronounced. A similar observation was made on proliferative responses of lymphocytes from BCG vaccinated human subjects to the 38 kD antigen (Young *et al.*, 1986).

It was found that Rb71 anti-Id not only elicited responses in primed animals but also sensitized mice for antigen specific DTH and proliferative responses. Thus, spleen cells from mice immunized with affinity purified Rb71 anti-Id showed a proliferative response to the 38 kD protein antigen of similar magnitude to that obtained from MTSE primed mice. However, Rb71 injected mice failed to produce an 'Ab3' idiotype-positive serum response (Praputpittaya, 1986), indicating that the Rb71 anti-Id stimulates T cells but not B cells of corresponding Id or paratope specificity.

It has been previously shown that the antibody levels to the TB71 epitope of the 38 kD antigen of *M. tuberculosis* were higher in H-2^b and H-2^d than in H-2^k mice (Ivanyi & Sharp, 1986). However, DTH reactions to the 38 kD antigen in three strains of mice primed with Rb71 did not follow an H-2 related hierarchy since the foot-pad responses in all tested strains of mice were of similar magnitude. The difference in response between anti-Id and antigenic stimulation may be attributed either to the participation of distinct T cell subsets (i.e. T helper versus T-DTH) or to a bypass of Ir gene control as reported previously for antibody responsiveness to poly (glu-Ala-Tyr) antigen (Roth *et al.*, 1985).

The internal image functions of Rb71 anti-Id could be interpreted in terms of mechanisms which determine the immunodominance of B and T cell stimulatory epitopes of protein antigens and anti-Id antibodies. It is generally believed that 'hapten' and 'carrier' like structures are mutually exclusive (Benjamin *et al.*, 1984). This is expected to be the outcome of the distinct recognition mechanisms operated by B and T cells respectively. Consequently, idiotypic domains within the combining site of the majority of antibodies would not be shared with structures within the T cell receptor. However, it is conceivable that in certain instances antibodies can be directed towards the

same epitope which is also presented in association with MHC class II molecules to T cells. In this case the antibody combining site and the T cell receptor may contain a common idiotype or a structurally similar domain which is complementary for the same epitope of the antigen. This explanation for the internal image activities of Rb71 for T cells could be investigated by further structural and functional analysis of the 38 kD antigen and of the Rb71 anti-Id determined idiotype.

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REFERENCES

BENJAMIN, D.C., BERZOFSKY, J.A., EAST, I.J., GURD, F.R.N., HANNUM, C., LEACH, S.J., MARGOLIASH, E., MICHAEL, J.G., MILLER, A., PRAGER, E.M., REICHLIN, M., SERCARZ, E.E., SMITH-GILL, S.J. TODD, P.E. & WILSON, A.C. (1984) The antigenic structure of proteins: a reappraisal. *Ann. Rev. Immunol.* **2,** 67.

BINZ, H. & WIGZELL, H. (1975) Shared idiotypic determinants on B and T lymphocytes reactive against the same antigenic determinants. I. Demonstration of similar or identical idiotypes on IgG molecules and T-cell receptors with specificity for the same allotypes. *J. exp. Med.* **142,** 197.

COATES, A.R.M., ALLEN, B.W., HEWITT, J., IVANYI, J. & MITCHISON, D.A. (1981) Antigenic diversity of *Mycobacterium tuberculosis* and *Mycobacterium bovis* detected by means of monoclonal antibodies. *Lancet* **ii,** 167.

COLLINS, F.M. & MACKANESS, G.B. (1968) Delayed hypersensitivity and arthus reactivity in relation to host resistance in Salmonella-infected mice. *J. Immunol.* **101,** 830.

ENGERS, H.D. & WORKSHOP PARTICIPANTS (1986) Results of a World Health Organization-sponsored workshop to characterize antigens recognized by *Mycobacterium*-specific monoclonal antibodies. *Infect. Immun.* **51,** 718.

ERTL, H.C.J., GREENE, M.I., NOSEWORTHY, J.H., FIELDS, B.N., NEPON, J.T., SPRIGGS, D.R. & FINBERG, R.W. (1982) Identification of idiotypic receptors on reovirus specific cytolytic T cells. *Proc. natn Acad. Sci. USA.* **79,** 7479.

IVANYI, J. (1986) Pathogenic and protective interactions in mycobacterial infections. *Clin. Immunol. All.* **6,** 127.

IVANYI, J., MORRIS, J.A. & KEEN, M. (1985) Studies with monoclonal antibodies to mycobacteria. In: *Monoclonal Antibodies AGainst Bacteria* (eds A.J.L. Macario & E.C. Macario, p. 59. New York. Academic Press.

IVANYI, J. & SHARP K. (1986) Control by H-2 genes of murine antibody responses to protein antigens of *M. tuberculosis.* Immunology **59,** 329.

KENNEDY, R.C., EICHBERG, J.W., LANFORD, R.E. & DREESMAN, G.R. (1986) Anti-idiotypic antibody vaccine for type B viral hepatitis in chimpanzies. *Science* **232,** 220.

KRONENBURG, M., KRAIG, E., SIU, G., KAPP, J.A., KAPPLER, J., MARRACK, P., PIERCE, C.W. & HOOD, L.E. (1983) Three T-cell hybridomas do not contain detectable heavy chain variable gene transcripts. *J. exp. Med.* **151,** 896.

LAGRANGE, P.H., MACKANESS, G.B. & MILLER, T.E. (1974) Potentiation of T cell-mediated immunity by selective suppression of antibody formation with cyclophosphamide. *J. exp. Med.* **139,** 1529.

MARCHALONIS, J.J. (1985) Antigen-binding molecules of T cells: distinction from MHC-restricted molecules and segmental homology to immunoglobulin V_H and T-cell receptor genes. *Scand. J. Immunol.* **21,** 99.

MCNAMARA, M.K., WARD, R.E. & KOHLER, H. (1984) Monoclonal idiotype vaccine against *Streptococcus pneumoniae* infection. *Science* **226,** 1325.

MITSOUKA, A., BABA, M. & MORIKAWA, S. (1976) Enhancement of delayed hypersensitivity by depletion of suppressor T cells with clclophosphamide in mice. *Nature.* **162,** 77.

PRAPUTPITTAYA, K. (1986) Anti-idiotypic antibodies and their role in the immune response to *M. leprae* and *M. tuberculosis.* PhD Thesis, University of Birmingham.

PRAPUTPITTAYA, K. & IVANYI, J. (1987) Study of idiotypes expressed by monoclonal antibodies to the 35 kD and 12 kD antigens of *Mycobacterium leprae. Clin. exp. Immunol.* **70,** 298.

RAJEWSKY, K. & EICHMANN, K. (1977) Antigen receptors of T helper cells. *Contemp. Top. Immunobiol.* **7,** 69.

RAMSEIER, H., AGUET, M. & LINDEMANN, J. (1977) Similarity of idiotypic determinants of T- and B-lymphocyte receptors for alloantigens. *Transplant. Rev.* **34,** 50.

REES, A.D.M., PRAPUTPITTAYA, K., SCOGING, A., DOBSON, N., IVANYI, J., YOUNG, D. & LAMB, J.R. (1987) T-cell activation by anti-idiotypic antibody: evidence for the internal image. *Immunology* **60,** 389.

ROTH, C., SOMME, G., GOUGEON, M.L. & THEZE, J. (1985) Induction by monoclonal anti-idiotypic antibodies of an anti-poly (glu^{60} Ala^{30} Tyr^{10}) (GAT) immune response in GAT-responder and GAT-nonresponder mice. *Scand. J. Immunol.* **21,** 361.

SACKS, D., ESSER, K.M. & SHER, A. (1982) Immunisation of mice against African trypanosomiasis using anti-Id antibodies. *J. exp. Med.* **155,** 1108.

SHARPE, A.H., GAULTON, G.N., MCDADE, K.K., FIELDS, B.N. & GREENE, M.I. (1984) Syngeneic monoclonal antiidiotype can induce cellular immunity to reovirus. *J. exp. Med.* **160,** 1195.

Thomas, W.R., Morahan, G., Walker, I.D. & Miller, J.F.A.P. (1981) Induction of delayed-type hypersensitivity to azobenzenearsonate by a monoclonal anti-idiotype antibody. *J. exp. Med.* **153,** 743.

YOUNG, D.B., KENT, L., REES, A., LAMB, J. & IVANYI, J. (1986) Immunological activity of a 38-kd protein purified from *Mycobacterium tuberculosis*. *Infect. Immunity* **54,** 177.

YOUNG, R.A., BLOOM, B.R., GROSSKINSKY, C.M., IVANYI, J., THOMAS, D. & DAVIS, R.W. (1985) Dissection of *Mycobacterium tuberculosis* antigens using recombinant DNA. *Proc. natn Acad. Sci USA*. **82,** 2583.