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The T-cell mediated immune response to *Schistosoma mansoni*

I. GENERATION OF STAGE-SPECIFIC, MHC-RESTRICTED PROLIFERATIVE T-CELL CLONES TO SOLUBLE EGG ANTIGENS

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Summary. A panel of T-cell clones reactive to the soluble egg antigen (SEA) of *Schistosoma mansoni* is described. The proliferative responses of the primary immune lymph node (LN) cells and T-cell clones to different schistosome antigen preparations (cercarial extract (CE), live or dead schistosomula, adult worm extract, and soluble egg antigen) were compared. Primary immune LN cells could not distinguish between these schistosome antigen preparations. However, when a total of 22 T-cell clones was analysed, a complex pattern of both stage-specific and common (or cross-reactive) antigen reactivity was observed. These patterns have been grouped into four types:

(i) five clones were reactive to all different schistosome antigen preparations;

(ii) two clones were reactive only to SEA, CE and schistosomula;

(iii) three clones were reactive only to SEA and cercariae;

(iv) twelve clones were reactive to SEA antigen alone.

The T-cell clones were identified as Thy-1-positive cells. All the clones were able to respond to exogenous IL-2 after antigenic stimulation. However, there was a variable degree of IL-2 responsiveness when compared with antigen-specific stimulation. Four T clones were selected for further studies on the genetic control of the proliferative response to schistosome antigens. One of the proliferative T clones was restricted by the IA subregion of the major histocompatibility complex (MHC), and the other three clones are restricted by the IE (A_eE_α) subregion.

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INTRODUCTION

A large body of evidence suggests that T lymphocytes are involved in the induction of resistance to challenge infection and in the development of granulomatous response to egg antigens of *S. mansoni* (Phillips *et al.*, 1983; Chensue, Wellhausen & Boros, 1981; Weinstock & Boros, 1983). Deprivation of T cells by thymectomy resulted in the reduction of resistance to challenge infection in permissive hosts (Doenhoff & Long, 1979) or non-permissive hosts (Cioli & Dennert, 1976). Reconstitution of athymic nude mice with thymus-derived lymphocytes resulted in (i) enhanced resistance to challenge infection (Sher *et al.*, 1982), and (ii) increased eosinophilia and the development of hepatic granulomas (Phillips *et al.*, 1977). Early studies suggested that antigen preparations prepared from different stages of schistosomes showed extensive serological cross-reactivity (reviewed by Damian, 1984). Application of the technology for the production of monoclonal antibodies has lead to the identification of stage-specific antigens on schistosomula (Tavares *et al.*, 1984) and adult worms (Mitchell *et al.*, 1981), and

the detection of cross-reactive (common) antigen on schistosomula, adult worm and miracidia (Smith *et al.*, 1982).

Recent studies show that adult worm antigen and incubation products of living adult schistosomes displayed extensive cross-reactivity when tested on the proliferative response of immune lymph node cells to these antigens (Louis *et al.*, 1984). Thus, despite the stage-specificity identified with monoclonal antibodies, there has been little clear evidence suggesting that T cells recognize stage-specific schistosome antigens.

In this study, clonal analysis of T-cell reactivity to different schistosome antigens was performed. The results supported and further extended previous observations to show that both common (or cross-reactive) antigen(s) and stage-specific antigen(s) could be detected by using cloned T cells. Furthermore, there is little information on MHC-controlled T-cell responses to parasites. This panel of T-cell clones provided an opportunity to investigate the details of MHC control of T cells to schistosome antigens. Surprisingly, a majority of clones are I-E (A_eE_α) restricted.

MATERIALS AND METHODS

Mice

Male CBA(H-2^k), B10A.3R(IE^k), and B10A.5R (IJ^k,IE^k) mice, 8–12 weeks old, were bred at this Institute. Male ATL (H-2^k), B10A.2R(K^k,IA^k,IJ^k,IE^k) and B10A.4R(K^k,IA^k) mice were obtained from the Imperial Cancer Research Fund, Mill Hill, London.

Culture conditions

All cells were cultured in RPMI-1640 (Gibco, Uxbridge, Middlesex) containing bicarbonate and supplemented with L-glutamine (2 mM final concentration), pyruvate (0·1 mM), penicillin (100 units/ml), streptomycin (100 μg/ml), HEPES (10 mM), and mono-thioglycerol ($7{\cdot}5 \times 10^{-5}$ M, Sigma, Poole, Dorset). Except where otherwise stated, 10% pretested fetal calf serum (FCS) was used for the cloning and cell proliferation assay. Cultures were incubated at 37° in 5% CO_2 in a humidified incubator.

Preparation of schistosome antigens

A Puerto Rican strain of *Schistosoma mansoni*, which is routinely maintained in snails (*Biomphalaria glabrata*) by Dr S. R. Smithers in this Institute, was used for this study. Cercarial extract (CE) and adult worm antigen preparations (AW) were prepared by six cycles of freezing in liquid nitrogen, followed by thawing at 37°. Large particles were removed by centrifugation (100 *g* for 10 min). Mechanically transformed schistosomula were prepared as described previously (Ramalho-Pinto *et al.*, 1974). Except where otherwise stated, freeze-thaw killed whole schistosomula were used for this study. Soluble egg antigen (SEA) was prepared by homogenizing the eggs in RPMI-1640 medium. In some experiments, the SEA used was kindly provided by the World Health Organization. All antigen preparations (CE, AW and SEA) were sterilized by membrane filtration (0·45 μm) and stored at −20°.

IL-2 source

IL-2-containing medium was prepared from EL4 lymphoma cells (Farrar *et al.*, 1980). The cells (10^6/ml) were treated with 100 ng/ml of phorbol myristate acetate (PMA) for 6 hr in RPMI medium supplemented with 2% FCS. The cells were then washed three times in RPMI medium and resuspended in RPMI (10^6/ml) containing 5% FCS. Cell-free supernatant was harvested at 24 hr after incubation and the supernatant was sterilized by membrane filtration. The amount of IL-2 activity present in the supernatant was determined in a standard microassay using an IL-2-dependent line (CTLL) as indicator cells (Gillis *et al.*, 1978). The reciprocal dilution of the supernatant which supported 50% maximum proliferation of the CTLL cells was defined as 1 unit. A final concentration of 30–40 units/ml of IL-2 was used for T cell cloning experiments.

Generation and propagation of T-cell clones

Immune spleen cells (1×10^6 cells/ml) from CBA mice, 7 weeks after infection with 100 cercariae, were stimulated with SEA (50 μg/ml) in 25-cm^2 flasks for 3 days. T cell cloning was performed by the limiting dilution method (Vitiello & Sherman, 1983). Various numbers of lymphoblasts (0·3–100 per well) were cultured with 2×10^5 irradiated syngeneic spleen cells, SEA (50 μg/ml) antigen, and IL-2 in 96-well round-bottomed plates for 9–14 days, and the proliferating cell frequency was estimated by maximum likelihood method (Taswell, 1981). Proliferating cells from plates containing less than 30% positive wells were expanded for further studies. The cells were transferred to, and maintained, in 24-well cluster plates (Costar, Cambridge, Cambs) containing 1×10^6 γ-irradiated spleen cells, antigen and IL-2. The cells were maintained by weekly restimulation with antigen.

Proliferation assay

Various numbers of responding cells were cultured in duplicate or triplicate with different concentrations of schistosome antigens and γ-irradiated normal spleen cells in 0·1 ml of medium in 96-well round-bottomed plates at 37° in 5% CO_2 for 48 hr (in the case of T-cell clones) or for 72 hr (in the case of primary LN cells). Medium containing 1% syngeneic mouse serum was used for the proliferation assay of primary immune LN cells. Cell proliferation was assayed by adding [^{3}H]methyl-thymidine ([^{3}H]TdR, 0·5 μCi/well, specific activity 5 Curies/mmole, Amersham International, Amersham, Bucks) for 16 hr. The contents of the wells were harvested onto glass fibre filters and processed for β-counting. For comparison between different experiments, results are presented as a stimulation index (SI) calculated from counts obtained when responding T cells and feeder cells were cultured with antigens or IL-2, divided by counts obtained from the same cell preparation when cultured in the absence of antigen or IL-2.

Immunofluorescent staining

Cell surface membrane antigens were determined by indirect immunofluorescence assay. Rat anti-mouse Thy-1 monoclonal antibody (NIM-R1) (Chayen & Parkhouse, 1982), and anti-Lyt-1 and anti-Lyt-2 monoclonal antibody (Ledbetter & Herzenberg, 1979) were used for this study. The fluorescein-conjugated rabbit anti-rat Ig was purchased from Sera Labs, Crawley Down, Sussex.

RESULTS

Proliferative response of primary immune LN cells

LN cells from CBA mice 1 week after cercarial infection were examined for their reactivity to different schistosome antigens. In agreement with previous studies (Lewis & Wilson, 1982), immune LN cells were able to proliferate in response to CE, AW or SEA antigen preparations. Results in Fig. 1 show that the optimum concentration of CE, AW and SEA for the proliferation assay was about 100, 50 and 25 μg/ml, respectively. Primary immune LN cells from mice infected with γ-irradiated cercariae showed similar cross-reactivity (data not shown). Incubation of the LN cells with high concentrations of AW or SEA resulted in reduced [^{3}H]TdR uptake.

Reactivity of schistosome-specific T-cell clones

Cell cloning was performed on Day 3 after stimulation of spleen cells (7 weeks after infection) with SEA. A total of 49 clones were originally isolated; about half of these died during expansion, leaving 22 clones to study in detail. All the clones studied displayed Thy-1

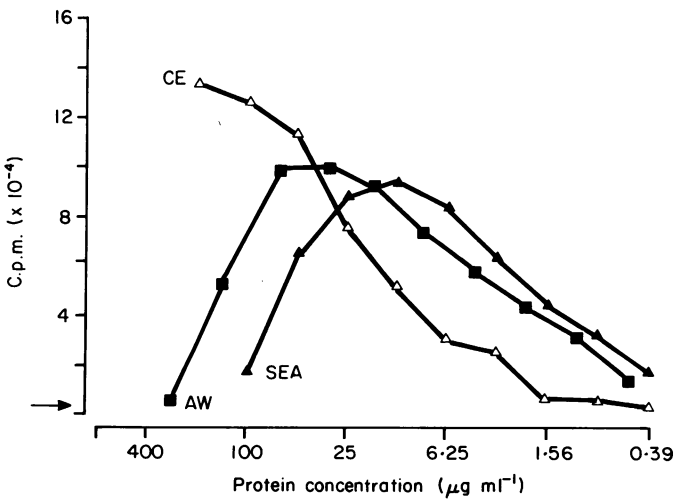

Figure 1. Reactivity of primary immune LN cells to different antigen preparations. LN cells (from mice 1 week after infection with 100 cercariae) were cultured with CE (△), AW (■) or SEA (▲) antigen for 3 days. The arrow (→) indicates the background [^{3}H]TdR uptake by the cells in the absence of antigen.

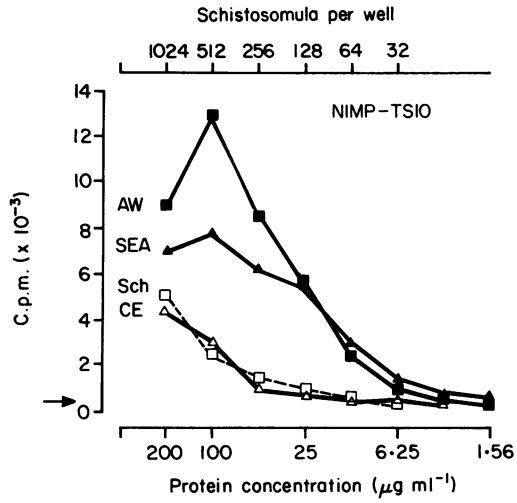

Figure 2. Type 1 antigen reactivity. Clone NIMP-TS10 reacted with AW (■), SEA (▲), schistosomula (□) or CE (△) antigens. The arrow (→) indicates the background uptake by the cells in the absence of antigen.

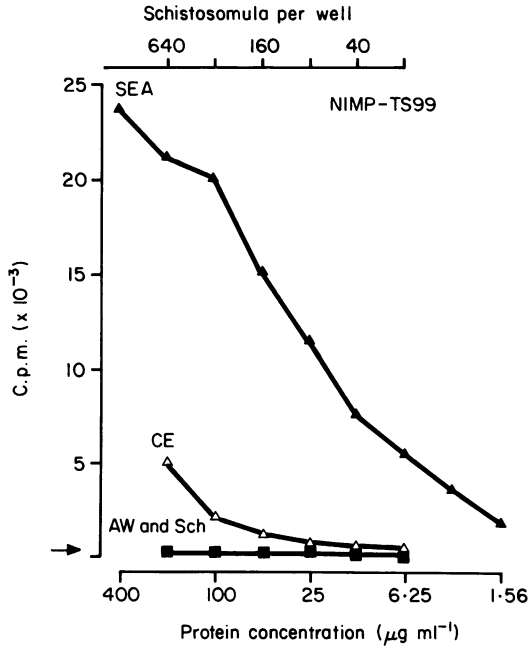

Figure 4. Type 3 antigen reactivity. Clone NIMP-TS99 reacted to SEA and with a weak reaction to CE. Symbols as in Fig. 2.

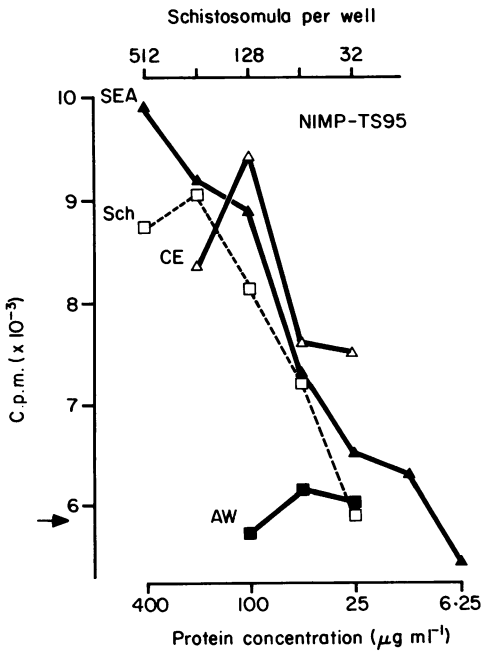

Figure 3. Type 2 antigen reactivity. Clone NIMP-TS95 reacted to all except AW. Symbols as in Fig. 2.

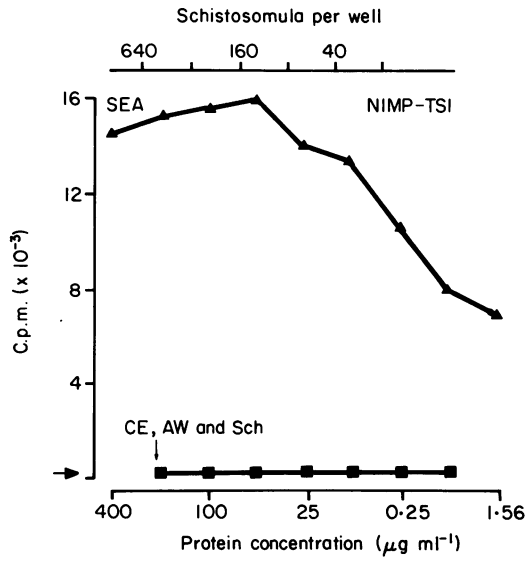

Figure 5. Type 4 antigen reactivity. Clone NIMP-TS1 reacted only to SEA. Symbols as in Fig. 2.

antigen when an indirect immunofluorescent staining method was used (data not shown). In view of the inhibition of cell proliferation by AW or SEA at high concentration, the antigen reactivity by T-cell clones was examined over a range of antigen concentrations (from 400 μg/ml to less than 1 μg/ml). Four patterns of antigen reactivity by these T-cell clones were observed.

Type 1. Five SEA-derived T-cell clones were found to be reactive to all antigen preparations (CE, schistosomula, AW and SEA). NIMP-TS10 is shown as an example in Fig. 2. This clone responded not only to killed schistosomula, but also to live schistosomula (data not shown).

Type 2. Two SEA-derived clones reacted to CE, schistosomula and SEA, but not to AW. NIMP-TS95 is shown as an example in Fig. 3.

Type 3. Three clones reacted to CE and SEA but not to schistosomula or AW. NIMP-TS99 is shown as an example in Fig. 4.

Type 4. Twelve SEA-derived clones reacted only to SEA, but not to other schistosome antigens. NIMP-TS1 is shown as an example in Fig. 5.

These results clearly show that T-cell clones with specificity for SEA antigen, and for both SEA and for antigens prepared from different stages of schistosomes, could be produced by stimulation with SEA. It should be noted that, within each of these types, the magnitude of the response of different clones to the same antigen preparation varied, suggesting that different clones may be reacting to different antigens.

The MHC-restriction of schistosome-specific T clones

Four schistosome-specific clones were selected for this

Table 1. MHC-restricted schistosome-specific T-cell clones*

Clone	APC†	Antigen (SEA)‡	MHC shared	Proliferative response ([^{3}H]TdR uptake)
NIMP-TS67	–	–	–	80±20
	–	+	–	158±26
	CBA	–	All	1004±286
	CBA	+	All	8094±741
	ATL	–	I	1225±313
	ATL	+	I	8252±270
	B10A.4R	–	K, IA	567±270
	B10A.4R	+	K, IA	6468±622
NIMP-TS99	–	–	–	222±37
	–	+	–	3175±574
	CBA	–	All	1987±206
	CBA	+	All	21,440±786
	ATL	–	I	3091±1033
	ATL	+	I	17,550±4207
	–	–	–	171±50
	–	+	–	2782±282
	CBA	–	All	1728±226
	CBA	+	All	33,873±2410
	B10A.2R	–	K, I-A, J, E	1156±226
	B10A.2R	+	K, I-A, J, E	23,512±923
	B10A.3R	–	IE	1369±516
	B10A.3R	+	IE	4573±276
	B10A.4R	–	K, IA	1074±193
	B10A.4R	+	K, IA	3363±159
	B10A.5R	–	IJ, IE	1524±67
	B10A.5R	+	IJ, IE	3700±184

Results are expressed as mean±SD from triplicate cultures.
* T-cell clones were tested 7 or 8 days after antigen stimulation.
† Antigen-presenting cells (APC) were irradiated normal spleen cells.
‡ SEA, 100 μg/ml.

study. Table 1 shows the MHC restriction pattern of two clones, NIMP-TS67 and NIMP-TS99. Immunofluorescent staining revealed that both of them were Thy-1$^+$ and Lyt-2$^-$. NIMP-TS67, which is a cross-reactive clone (reactive to CE, AW, SEA and schistosomula), responded to SEA antigen in the presence of I-region (ATL) or K/IA region (B10A.4R) histocompatible antigen-presenting cells. NIMP-TS99 responded to SEA antigen and antigen-presenting cells histocompatible for the K/IA, IJ, and IE subregion (B10A.2R). The sharing of the IE (B10A.3R), K/IA (B10A.4R) or IJ, IE (B10A.5R) subregion of the MHC between NIMP-TS99 and antigen-presenting cells is not effective at stimulating the antigen-specific proliferative response of this clone. The other two clones, NIMP-TS18 and NIMP-TS47, showed similar restriction patterns to NIMP-TS99 (data not shown). These results indicated that the activation of these schistosome antigen-specific T-cell clones is restricted by Class II MHC molecule.

IL-2 responsiveness of T-cell clones

In order to compare the ability of schistosome-activated T cells to respond to further IL-2 or antigenic restimulation, the cells were maintained in 30 units/ml of IL-2 for 7–8 days after antigen stimulation. The cells were washed and incubated with different concentration of SEA antigen (1–400 μg/ml) or IL-2-containing EL4 culture supernatant (0–320 units/ml). The results from four typical clones are shown in Table 2,

Table 2. Proliferative response of schistosome-specific T-cell clones to antigen or IL-2-containing medium

	Stimulation index*	
Clones	SEA	EL4 sup.†
NIMP-TS1	169·2	43
NIMP-TS10	42·6	46·7
NIMP-TS99	72·6	17·5
NIMP-TS35	98·1	233·2

* The value, which was obtained from the antigen titration curve, indicated the maximum antigen stimulation index.

† The same cell preparations were stimulated with 320 units per ml of IL-2-containing EL4 cell culture supernatant.

and only the data from the maximum antigen stimulation are presented. All antigen-activated T-cell clones are able to respond to exogenous IL-2. When compared with antigen-specific restimulation, the magnitude of the proliferative response of the same clone to IL-2 or to antigen varied from one clone to the next. For example, NIMP-TS1 responded more strongly to antigen than to IL-2, whereas NIMP-TS95 responded more strongly to IL-2 than to antigen.

DISCUSSION

Previous studies using the lymphocyte proliferative assay showed that primary immune LN cells or spleen cells were highly reactive to soluble schistosome antigens or living schistosomula (James, 1981; Lewis & Wilson, 1982). The results presented in this paper support the observation (Lewis & Wilson, 1982) that LN cells, obtained from mice as early as 1 week after infection, recognized cercarial, adult worm, and egg antigen preparations, although, clearly, the animal would not be exposed to egg antigens until 5–6 weeks after infection. These results give no indication to suggest that T cells recognize stage-specific antigens. Recently, T-cell clones specific for the incubation products of *S. mansoni* adult worm were produced (Louis *et al.*, 1984), but no details of the stage-specificity of these clones were presented.

To examine the T-cell reactivity in more detail, a panel of SEA-reactive T-cell clones was derived and characterized. In order to minimize the selection of T-cell clones during long-term bulk culture, the cell cloning procedures were carried out 3 days after primary *in vitro* stimulation of immune spleen cells. In view of the possibility of the loss of antigen specificity by long-term cultured T-cell clones as reported elsewhere (Simon *et al.*, 1984), the antigen-specificity tests were performed in the first 2 months after the establishment of T-cell clones.

In order to determine the pattern of antigen reactivity, the proliferative response was determined by titrating the responder cells against a range of antigen concentrations. A complex pattern of antigen reactivity was observed. However, four patterns emerge which simplify the presentation of these data:

(i) antigen common to all stages;

(ii) antigen detectable in all stages except adult worm antigen preparation;

(iii) antigen present in SEA and cercarial antigen preparation but undetectable in other stages;

(iv) antigen present in SEA but undetectable in other stages.

It must be emphasized that, within each type, there is likely to be reactivity to more than one antigen, and that other patterns will probably emerge as more T-cell clones are analysed. The details of antigen specificity will become clear only when the individual antigens involved are identified. However, a classification of this type should make it easier to compare these results with biochemical and antibody analyses. It is of interest to note that 12 out of 22 clones reacted with antigens present only in SEA preparation, the remaining SEA-derived clones also reacted with antigens prepared from different stages of schistosome. The presence of these cross-reactive proliferative clones may explain the previous observation by Lewis & Wilson (1982) and the cross-reactive nature of primary immune LN cells as reported in this paper. The SEA-specific T-cell clones should provide a useful tool to study the modulation of egg-granuloma formation by T cells and facilitate the further identification of egg antigen(s) which induces the granuloma hypersensitivity reaction.

It had been demonstrated that activated T cells acquired responsiveness to IL-2 after activation by antigen (Larsson, 1981), and the proliferative response of activated T cells to IL-2 is due to the modulation of the expression of IL-2 receptor by antigen (Andrew, Braciale & Braciale, 1984; Helmer *et al.*, 1984). After antigen or lectin stimulation, there is an increase in IL-2 receptor expression followed by a slow decay. The ability of the cells to proliferate in response to IL-2 decayed with the same kinetics as IL-2 receptor levels (Cantrell & Smith, 1983; Kaplan, Braciale & Braciale, 1984). Thus, the periodic restimulation of T cells with specific antigen and IL-2 is necessary for the long-term maintenance of T-cells. The present results emphasize the heterogeneity between clones in their relative ability to respond to IL-2 or antigen 8 days after stimulation with antigen. For example, NIMP-TS1 (Table 2) shows a limited response to IL-2 but a massive response to antigen, suggesting that the expression of IL-2 receptors has waned. In contrast, clone NIMP-TS35 shows a much greater ability to respond to IL-2 than to antigen. From the practical point of view, these results indicate that the requirements for antigen restimulation and IL-2 concentration are different for individual clones. A better understanding of these factors would allow a better assessment of the growth requirements of individual clones, and might allow a higher proportion of clones to be maintained.

Recognition of foreign antigen in association with self MHC molecules is one of the major characteristics of T cells (Zinkernagel & Doherty, 1979). The type of T-cell response (e.g. helper or suppressor T cells) to a particular foreign antigen seems to be dependent on the class of MHC molecules which T cells recognize (Ada, Leung & Erth, 1981). Thus, the class of MHC molecules involved in the antigen presentation may determine the outcome of the host immune response (Blanden, 1980). In the case of schistosome infection, the development of vaccine-induced resistance in inbred mice is influenced by genetic differences at the MHC locus (Sher, Hieny & James, 1984). Furthermore, IC subregion-encoded soluble suppressor factor, which was produced by Lyt-2,3$^+$, I-J$^+$/I-C$^+$ T suppressor cells, appears to play a role in the regulation of granulomatous inflammation in murine schistosomiasis (Chensue, Boros & David, 1983). These results indicate that MHC molecules might play an important role in controlling the host immune response to schistosome infection.

It has become clear that I-region restricted T cells can be restricted by I-E (A_eE_α) or I-A ($I_\beta A_\alpha$) molecules (Schwartz, Chen & Paul, 1980). A wide variety of antigens are able to be presented to T cells in association with either I-A or I-E molecules (Marusic *et al.*, 1982). Although these experiments were not carried out at the clonal level, it suggests that I-region restricted T cells responding to most antigens are restricted by either IA or IE molecule. The exception in this work was that T cells from responder strains which respond to *Blastomyces* antigen were restricted by IA only. The implication of most of the limited information on the relative importance of I-A compared to I-E restriction elements appears to be that most T cells to most antigens are I-A restricted. For example, Conrad & Janeway (1984) added monoclonal anti-I-A to increase the frequency of I-E restricted clones to ovalbumin. On the other hand, the proliferative response of T cells to pigeon cytochrome *c* (Matis *et al.*, 1982), and amino acid terpolymers (Nagy *et al.*, 1981) are I-E restricted, suggesting a link between certain antigens and certain restriction elements. Krco *et al.* (1983) described two T-cell clones responding to *Trichinella spiralis* antigens. Both were I-E restricted. However, these clones were isolated from long-term T-cell cultures so that selection *in vitro* could be taking place, making it difficult to assess the relative significance of I-A and I-E restriction elements in the total response. The clones responding to SEA in the present work were isolated after 3 days in bulk culture, so that

very little selection could be taking place. Three out of four clones recognize antigen presented in association with an I-E molecule. This, and Krco's work, point to the importance of the I-E molecule in the response to these parasitic diseases, and clearly, more work is required to understand the significance of these findings.

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