Heligmosomoides polygyrus superantigen: differential response with mouse and human lymphocytes

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(*Received 15 April 1997; revised 19 May 1997; accepted 21 May 1997*)

SUMMARY

The interaction between *Heligmosomoides polygyrus* superantigen and human peripheral blood mononuclear cells (PBMC) was evaluated. Parasite homogenate and excretory–secretory proteins from both L_4 larvae and adult worms were examined for their ability to stimulate, and be presented by, human cells. Proliferation assays using PBMC from 3 human volunteers indicated that naïve cells were stimulated by *H. polygyrus* superantigen. Antigen presenting cells (APC) from a number of human donors were able to successfully present *H. polygyrus* superantigen to mouse T cell hybridomas. However, this ability varied according to the source of the superantigen, and human APC, in contrast to APC from mice, could only present the superantigen contained within parasite homogenate. Also, in contrast to the situation with mice, human APC could present *H. polygyrus* superantigen to stimulate mouse T cells expressing not only TCR Vβ8.1, but also TCR Vβ7. Therefore, *H. polygyrus* superantigen can successfully stimulate, and be presented by, human PBMC of different MHC haplotypes, although the cellular mechanisms appear to be different from those observed in the mouse.

Key words: *Heligmosomoides polygyrus*, superantigen, human, mouse, APC.

INTRODUCTION

Heligmosomoides polygyrus is a nematode parasite of mice which has been extensively researched, in part, because of its ability to manipulate the host immune response (Behnke, 1987). The mechanisms involved in this regulation of host immunity have been investigated, although there is little consensus of opinion as to the actual processes involved, other than an ability of both larval and adult stages to manipulate the host immune system (Behnke, Hannah & Pritchard, 1983; Ali & Behnke, 1984). This immunomodulation can be demonstrated by both live parasites and extracted protein preparations (Pritchard, Ali & Behnke, 1984; Pritchard & Behnke, 1985; Crawford, Behnke & Pritchard, 1989).

We have previously demonstrated that *H. polygyrus* produces a superantigen-like molecule which has the ability to strongly stimulate naïve host T cells (Robinson *et al*. 1994). Like other exogenous superantigens, this stimulates only T cells bearing specific T cell receptor (TCR) Vβ-types (Kappler *et al*. 1989; Marrack & Kappler, 1990), and T cells bearing Vβ8.1 are stimulated by this superantigen while those bearing Vβ7 are not (Robinson *et al*. 1995). In addition, *H. polygyrus* superantigen, in common with bacterial superantigens, demonstrates a requirement for MHC-positive antigen presenting cells (APC), in order to stimulate T cells (Irwin & Gascoigne, 1993; Webb & Gascoigne, 1994). *H. polygyrus* superantigen has a unique requirement for MHC class I (Robinson *et al*. 1995), not MHC class II, although this MHC requirement, as is the case with many other superantigens (Gascoigne & Ames, 1991; Labrecque, Thibodeau & Sekaly, 1993), is not restricted to syngeneic molecules. In fact the *H. polygyrus* superantigen appears to be MHC promiscuous, and APC from mice with a wide range of H-2 haplotypes can present this molecule to the same T cells (Robinson *et al*. 1994).

As an MHC promiscuous molecule, which can strongly stimulate T cells, may be of more than just academic interest, we decided to determine whether *H. polygyrus* superantigen is able to interact immunologically with human cells, or whether these interactions are species specific.

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MATERIALS AND METHODS

Mice

B10·BR (H-2^k), C57BL/10 (H-2^b) and B10.D2 (H-2^d) mice were bred and maintained in the Department of Veterinary and Microbiological Sciences, NDSU. MHC class I deficient, β2m- mice (designated CID in the Fig. 4), were obtained from the Immunogenetics Mouse Colony, Mayo Foundation. Mice were a minimum of 2 months old at the time of experimentation.

The parasite

The production and maintenance of *H. polygyrus* was in outbred Swiss Webster mice, and as described by Jenkins & Behnke (1977).

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Antigen

For the preparation of 7-day-old L_4 worms, the small intestine of infected mice was removed and slit open, and the mucosal surface scraped off using the edge of a microscope slide. L_4 worms were then removed from the remainder of the gut, using the modified Baermann technique utilized for the preparation of adult worms (Jenkins & Behnke, 1977). The preparation of parasite homogenate has been previously described in detail (Robinson & Gustad, 1996). Excretory–secretory (E–S) proteins were produced by repeatedly washing parasites in sterile PBS, then incubating them in PBS, supplemented with penicillin (100 U/ml), streptomycin (100 μg/ml), for 24 h at 37 °C, in 5% CO_2. Using aseptic techniques, the supernatant was removed and centrifuged to remove debris. Protein concentrations were determined by spectrophotometry, as described below. Four parasite preparations were used: adult worm homogenate (AWH), adult worm excretory–secretory antigen (AWES), L_4 homogenate (L_4H), and L_4 excretory–secretory (L_4ES).

Staphylococcal enterotoxin

Staphylococcal enterotoxin B (SEB) was purchased from Sigma (St Louis, MO) and used at the concentration specified.

T cell hybridomas

T cell hybridomas IIA6 and IIB3 were produced in B10.BR mice and have been described previously. IIA6 expresses TCR Vβ8.1 and is stimulated by *H. polygyrus* superantigen in the presence of MHC class I-positive APC. Hybridoma IIB3 expresses Vβ7 and has been shown to be unresponsive to *H. polygyrus* superantigen in the presence of mouse antigen presenting cells (APC) (Robinson *et al*. 1995).

For the T cell hybridoma assays, 10^5 hybridoma cells were incubated with the stated amount of antigen for 2 days, using 4×10^5 irradiated APC. Supernatants were then removed and frozen at −135 °C for a minimum of 2 h, prior to incubation with 10^4 HT-2 cells, for 48 h. Assessment of HT-2 cell proliferation was by the uptake of [^{3}H]thymidine, as indicated previously (Robinson *et al*. 1994). During the stimulation of T cell hybridomas, irradiated splenocytes (mice) or peripheral blood mononuclear cells (PBMC; human) were used as APC, as indicated.

Proliferation assays

The procedures used to carry out a lymphocyte proliferation assay have been published previously (Robinson *et al*. 1994). Briefly, 5×10^5 mouse splenocytes or human PBMC were incubated with the indicated amount of AWH or staphylococcal enterotoxin B (Sigma) in DMEM, pH 7·2, supplemented with: glucose (4500 mg/l), $NaHCO_3$ (3·7 g/l), MOPS (10 mM), 2-ME (5×10^{-5} M), penicillin (100 U/ml), streptomycin (100 μg/ml), and 10% FCS, in sterile 96-well microtitre plates, incubated at 37 °C, in 5% CO_2. Assays were set up as triplicate or quadruplet wells and the proliferation of cells was assessed by an increase in viable cell numbers relative to that of controls, measured by the uptake of 0·625 μCi of [^{3}H]thymidine/well, over 18 h (specific activity 2 Ci/mmol). Assays were carried out for the number of days indicated.

RESULTS

To determine whether naïve human lymphocytes would be stimulated by the superantigen produced by *H. polygyrus*, human PBMC from 3 volunteer donors (TG, MM and JB) were stimulated by L_4ES (Fig. 1A), L_4H (Fig. 1B), AWH (Fig. 1C), and AWES (Fig. 1D). Naïve splenocytes from B10.D2 mice were similarly stimulated. The antigen preparation stimulated the proliferation of all of the different cell types with varying degrees of efficiency. AWES was a very poor stimulant for all species (Fig. 1D), while AWH was more efficient at stimulating cells from 2 of the humans (TG and JB) than those from B10.D2 mice (Fig. 1C).

As naïve cell proliferation assays are subject to many variables we decided to determine whether human cells could act as antigen presenting cells for *H. polygyrus* superantigen. We therefore utilized 2 T cell hybridomas, IIA6 and IIB3, as target cells. To determine whether these hybridomas could respond to superantigens presented by human cells, we initially used SEB as our stimulant and compared the response of the 2 hybridomas, when APC from donors TG and JB were used. As in Fig. 1, splenocytes from B10.D2 mice were utilized as a control. The results (Fig. 2) indicate that human cells successfully present superantigens to both of these hybridomas. Therefore, there was no species barrier to the use of human T cells as APC for these mouse T cell hybridomas.

The next series of experiments was carried out to determine whether human PBMC could present the superantigen in the 4 antigenic preparations, to T cell hybridomas IIA6 and IIB3. Surprisingly, the results obtained with human APC contrasted strongly with those obtained using APC from mice. Although the human APC varied in their ability to present superantigen, hybridomas IIB3 and IIA6 were both stimulated with approximately equal efficiency (Fig. 3A and B). In contrast, the mouse APC could not present superantigen to hybridoma IIB3 (Fig. 3C). Additionally, L_4ES antigen poorly stimulated the hybridomas when presented by

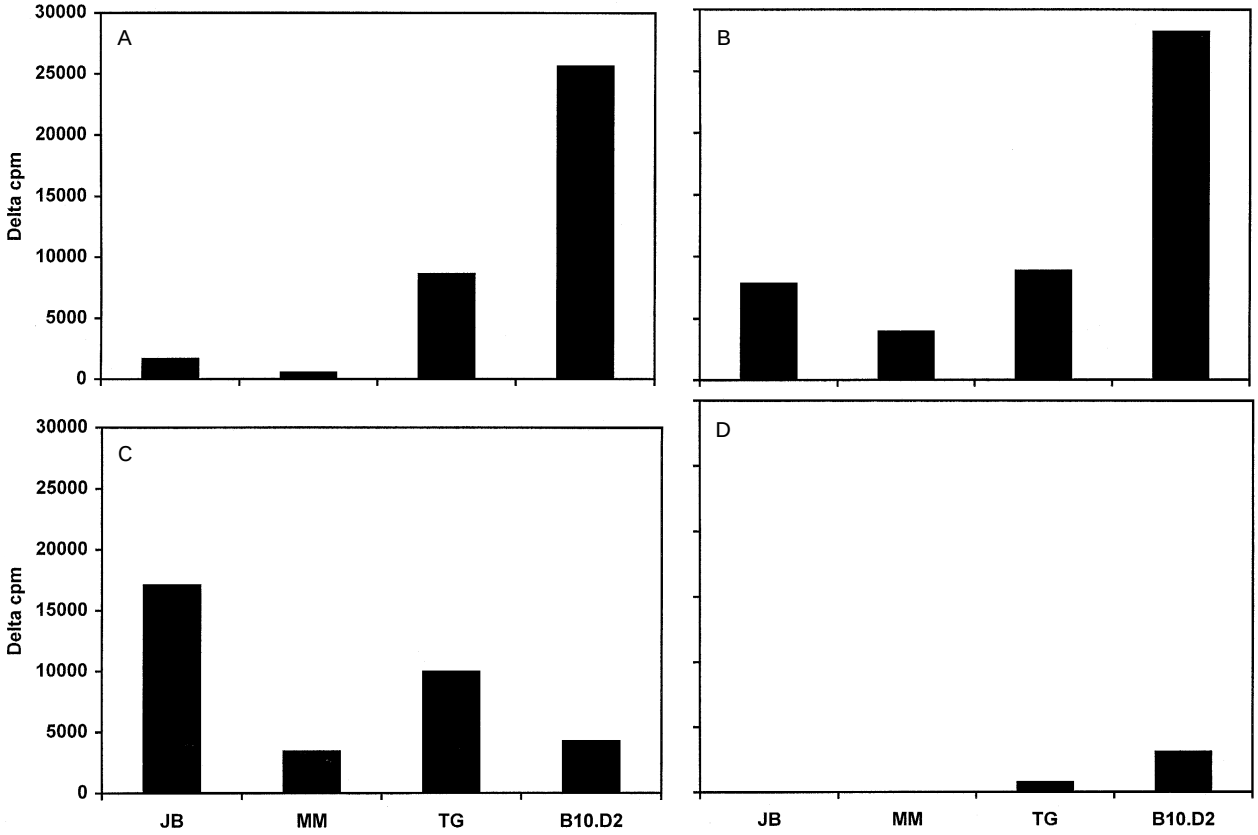

Fig. 1. The proliferative response of either human PBMC donors TG, MM and JB, or mouse (B10.D2) splenocytes to (A) 25 μg/ml L_4ES, (B) 25 μg/ml L_4H, (C) 100 μg/ml AWH and (D) 25 μg/ml AWES. The data indicate peak proliferation (usually day 5 or 6). Delta cpm = the mean of test wells minus control wells.

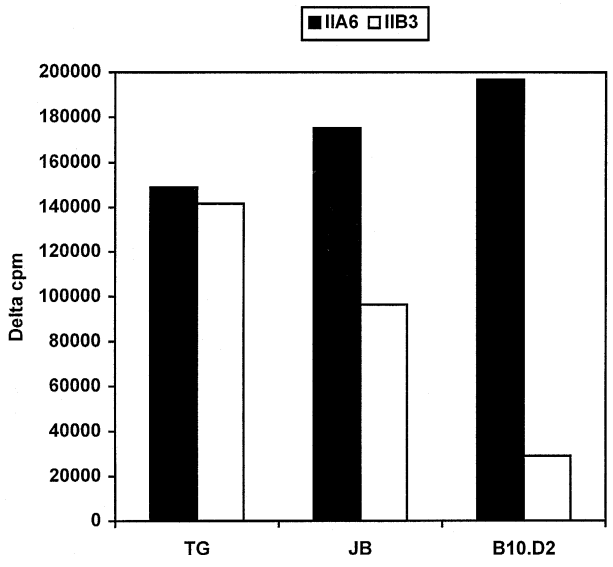

Fig. 2. The response of T cell hybridomas IIA6 and IIB3 to SEB (2 μg/ml). The data show the response of HT-2 cells to T cell hybridoma supernatants. Delta cpm = the mean of test wells minus controls.

human APC (Fig. 3A and B), in marked contrast to the situation seen with mouse APC, where L_4ES was a very strong stimulant (Fig. 3C).

To ascertain if the superantigen contained within L_4ES was atypical, an experiment was set up to demonstrate whether the superantigen in L_4ES could be presented by syngeneic (B10.BR) APC, APC from another allogeneic mouse strain (C57BL/10), or by MHC class I-deficient APC from β2m- mice. Even 5 μg/ml of L_4ES was strongly stimulatory for T cell hybridoma IIA6 when presented by syngeneic or allogeneic APC, but not when MHC class I-deficient APC were used (Fig. 4A). Conversely, APC from all strains could successfully present SEB to T cell hybridoma IIA6 (Fig. 4B).

The results obtained with the human APC had been rather unexpected, and as the superantigen presentation abilities of the 2 donors tried had been somewhat different, we extended this work to include APC from 3 further human volunteers, in addition to donor TG. L_4ES was used at a concentration of 5 μg/ml to obtain a comparison with the data shown in Fig. 4. The results obtained confirmed the previous observations, that AWH, and L_4H can successfully stimulate T cell hybridomas IIA6 and IIB3, when presented by human APC (Fig. 5). However, the ability of L_4ES to stimulate either of the T cell hybridomas was either poor or absent when presented by human APC.

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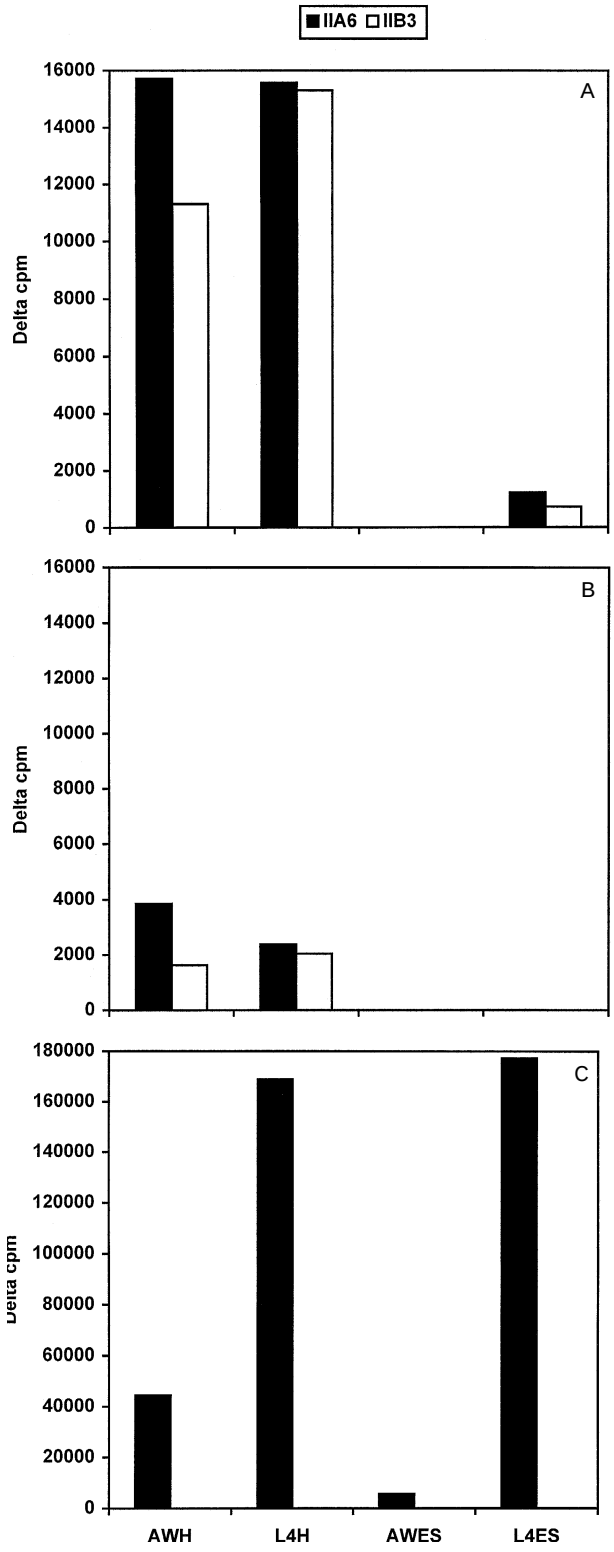

Fig. 3. The AWH (100 μg/ml), L_4H (100 μg/ml), AWES (25 μg/ml) or L_4ES (25 μg/ml), when presented by either human PBMC (A) donor TG, (B) donor JB or (C) B10.D2 splenocytes. The data show the response of HT-2 cells to T cell hybridoma supernatants. Delta cpm = the mean of test wells minus controls. Note that the range values for (A), (B) and (C) are not identical.

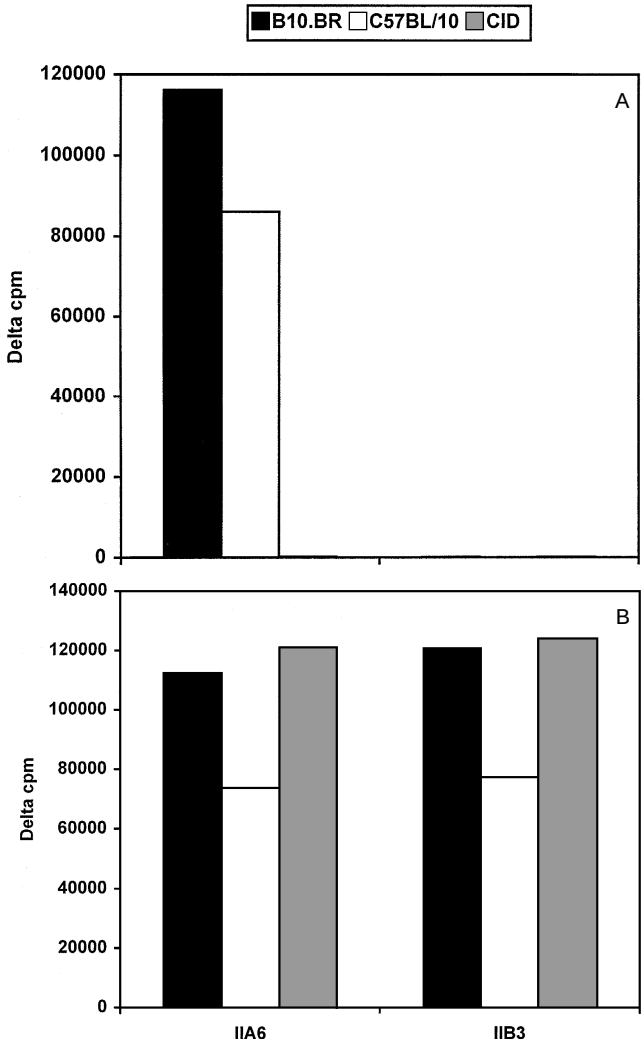

Fig. 4. The response of T cell hybridomas IIA6 and IIB3 to (A) L_4ES (5 μg/ml) and (B) SEB (2 μg/ml), when presented by APC from B10.BR, C57BL/10, or β2m- (CID) mice. The data show the response of HT-2 cells to T cell hybridoma supernatants. Delta cpm = the mean of test wells minus controls.

DISCUSSION

There are several points of interest presented in this work which extend our knowledge about the mechanism operating during the presentation of *H. polygyrus* superantigen. The first of these is that human cells appear able to be stimulated by the superantigen produced by *H. polygyrus*.

Also confirmed is that *H. polygyrus* superantigen, like SEB, can be presented by human APC to mouse T cells. This ability had also been demonstrated for the mouse mammary tumour virus superantigen, Mtv-7 (Subramanyam *et al.* 1993). Accordingly, the expectation of our original investigation has been confirmed, and a range of individuals from an outbred human population could successfully present the superantigen contained within parasite homogenate. Thus, these results extend the promiscuity of *H. polygyrus* superantigen across the species barrier. However, the response of cells to

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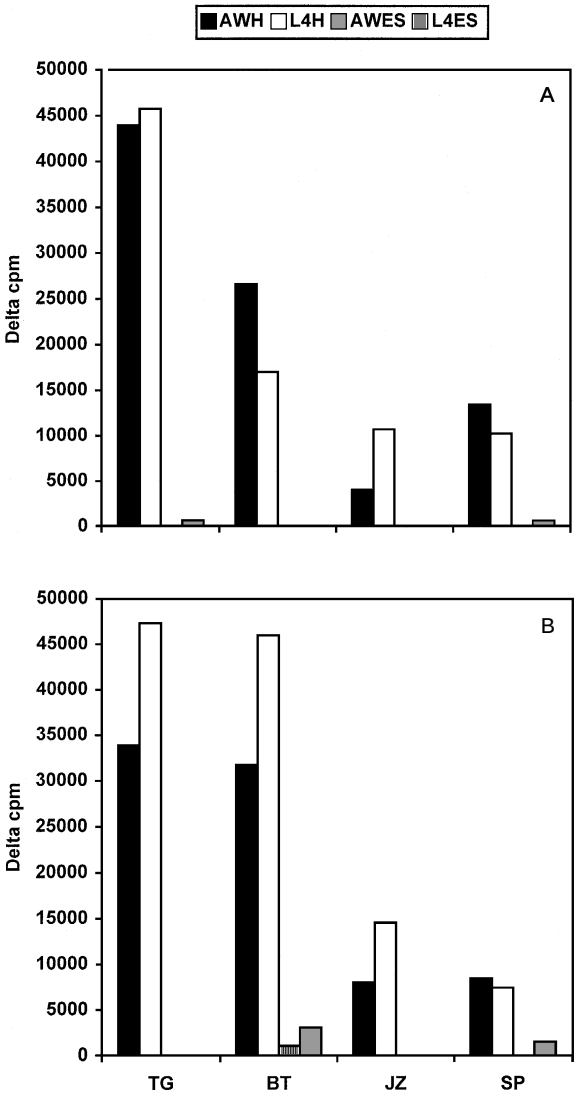

Fig. 5. The response of T cell hybridomas, (A) IIA6 and (B) IIB3 to AWH (100 μg/ml), L_4H (100 μg/ml), AWES (25 μg/ml), and L_4ES (5 μg/ml) when presented by PBMC from donor TG and additional donors BT, JZ, and SP. The data show the response of HT-2 cells to T cell hybridoma supernatants. Delta cpm = the mean of test wells minus controls.

superantigen contained within the 4 protein fractions utilized, appears to differ. We had previously shown that a superantigen is contained in both the E–S and homogenate protein fractions of adult and L_4 worms and that the concentration of superantigen appeared to be higher in the L_4ES protein preparation (Robinson *et al.* manuscript submitted). Here we confirm that the superantigen contained in L_4ES is at a high titre and, even at 5 μg/ml, is strongly stimulatory for T cell hybridoma IIA6, when presented by mouse APC. However, this strong stimulation does not occur when human APC are used. In fact both adult and larval E–S protein fractions were largely unable to stimulate T cell hybridomas at the concentrations used. Conversely, the superantigen contained in both AWH and L_4H was successfully presented by both human and mouse APC.

Another point of interest is that the superantigen contained within the protein fractions, most notably the homogenate preparations, is able to stimulate T cell hybridomas expressing TCR Vβ7. This is in contrast to the situation seen when using mouse APC, and which had confirmed our previous observations using AWH (Robinson *et al.* 1995).

We believe that there may be a number of ways to explain these discrepancies. The first is that there is a mitogen contained within the homogenate, perhaps resulting from contamination of the antigen preparation. This is, however, unlikely because it would also be presented by the mouse APC to hybridoma IIB3. As far as we are aware, there are no mitogens whose presentation to T cells follows this pattern. A second possibility is that there are different superantigens produced by *H. polygyrus* in each of the protein fractions. We cannot rule out this possibility, although again when presented by mouse APC, L_4ES and AWH have the same cellular requirements: for specific TCR Vβ, and MHC class I, while at the same time being promiscuous in their MHC requirements (Robinson *et al.* 1994, 1995). In addition we have confirmed that these requirements also extend to AWES (data not shown). Thus although it is possible that we are dealing with 4 completely different molecules, it is unlikely. However, it is possible that the molecules are modified in some way, which changes their cellular requirement. For instance, recombinant and purified SEB are known to behave slightly differently, in that purified forms activate TCR Vβ-types not stimulated with the recombinant form of the molecule (Herman *et al.* 1991). Therefore, it is conceivable that the superantigen is produced in modified forms, perhaps with varying degrees of glycosylation. This hypothesis would also be consistent with the fact that when the superantigen is presented by human APC, hybridoma IIB3, which had been formerly unresponsive with mouse APC, was now stimulated. Another possibility is that the combination of different MHC plus superantigen, stimulates different TCR Vβ-types. It has previously been shown with SEB, that presentation by MHC molecules of differing haplotype can extend the range of TCR Vβ-types stimulated (Surman *et al.* 1994; Wen, Blackman & Woodland, 1995). However, these results extrapolate this observation beyond a few additional haplotypes, as all of the human APC which could present superantigen to hybridoma IIA6 could also present to hybridoma IIB3. This indicates that in contrast to our previous observation (Robinson *et al.* 1995), the MHC is crucial in the cellular response to *H. polygyrus* superantigen.

In conclusion, these results show that human cells appear to be able to respond to *H. polygyrus* superantigen, and are able to present the super-

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antigen present in both AWH and L_4H to mouse T cells. However, other protein preparations known to contain superantigen when using mouse APC, notably L_4ES, were poorly stimulatory, at best, in the presence of human APC. Finally, *H. polygyrus* superantigen, when presented by human APC, was also stimulatory for T cell hybridomas expressing TCR Vβ7. These T cell hybridomas are not stimulated by *H. polygyrus* superantigen, when presented by mouse APC.

The authors acknowledge Dr Chella David, Department of Immunology, Mayo Foundation, for the β2m- mice, and for his help during the preparation of this manuscript. The Mayo Immunogenetics mouse colony is supported in part by NIH grant CA 24473. The authors also gratefully acknowledge the generous help of the volunteers who donated PBMC for this work.

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