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# Regulation of T cell response to leishmania antigens by determinants of histocompatibility leukocyte class I and II molecules

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## Abstract

It has been shown that HLA class I molecules play a significant role in the regulation of the proliferation of T cells activated by mitogens and antigens. We evaluated the ability of mAb to a framework determinant of HLA class I molecules to regulate T cell proliferation and interferon gamma (IFN-γ) production against leishmania, PPD, *C. albicans* and tetanus toxoid antigens in patients with tegumentary leishmaniasis and healthy subjects. The anti-major histocompatibility complex (MHC) mAb (W6/32) suppressed lymphocyte proliferation by 90% in cultures stimulated with αCD3, but the suppression was variable in cultures stimulated with leishmania antigen. This suppression ranged from 30-67% and was observed only in 5 of 11 patients. IFN-γ production against leishmania antigen was also suppressed by anti-HLA class I mAb. In 3 patients IFN-γ levels were suppressed by more than 60%, while in the other 2 cultures IFN-γ levels were 36 and 10% lower than controls. The suppression by HLA class I mAb to the proliferative response in leishmaniasis patients and in healthy controls varied with the antigens and the patients or donors tested. To determine whether the suppression is directed at antigen presenting cells (APCs) or at the responding T cells, experiments with antigen-primed non-adherent cells, separately incubated with W6/32, were performed. Suppression of proliferation was only observed when the W6/32 mAb was added in the presence of T cells. These data provide evidence that a mAb directed at HLA class I framework determinants can suppress proliferation and cytokine secretion in response to several antigens.

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- Tegumentary leishmaniasis
- Modulation of T cell response by HLA class I
- Interferon-γ and HLA class I
- Soluble antigen and HLA class I

## Introduction

T cell activation and proliferation in response to antigens is initiated by interactions between T cells and accessory cells which process and present antigens in association with membrane-bound histocompatibility antigen (HLA) molecules. The primary role of HLA class I molecules in T cell activation is the presentation of cytosolic antigens such as viral antigens to CD8+ T cells. More recently it has been shown that distinct determinants of HLA class I molecules may be associated with regulation of T cell responses to alloantigens, lectins and anti-CD3 (1,2). Little is known, however, about the participation of distinct determinants of HLA class I molecules in the regulation of antigen-specific T cell proliferation and cytokine production.

Taking advantage of our previous experience in the analysis of T cell responses in leishmaniasis (3,4) and since CD8+ T cells are expanded *in vitro* after stimulation with leishmania antigen (5), we evaluated the ability of a mAb to a framework determinant of HLA class I molecules to regulate T cell proliferation and IFN-γ production in response to leishmania, PPD, *C. albicans* and tetanus toxoid (TT) antigens in patients with tegumentary leishmaniasis and in healthy subjects. Additionally, it was observed that anti-major histocompatibility complex (MHC) class I antibodies modulate the immune response by T cells.

## Material and Methods

### Subjects

Eleven patients with tegumentary leishmaniasis (cutaneous or mucocutaneous leishmaniasis) were studied. The diagnosis was based on clinical manifestation of the disease and confirmed by histology and at least one of three tests: intradermal skin test to leishmania antigen, serological test and parasite isolation. Patients had active disease, and significant lymphocyte proliferative responses to leishmania, PPD, *C. albicans* and TT antigens. Six healthy subjects previously known to have reactive T cells to PPD, *C. albicans* and TT antigens were also studied.

### Mononuclear cell separation and proliferation assay

Peripheral blood mononuclear cells (PBMC) were isolated from heparinized blood by density gradient centrifugation using lymphocyte separation medium (Bionetics Laboratory Products, Kensington, MD, USA). After separation, the PBMC were collected from the interface and washed twice in sterile saline (0.9% NaCl).

The blastogenesis assay was performed as previously described (3). Briefly, PBMC were adjusted to a concentration of $10^6$ cells/ml in RPMI 1640 (Gibco, Grand Island, NY, USA) supplemented with 15% $AB^+$ heat-inactivated serum, and aliquots of 2 x $10^5$ cells were then cultivated in triplicate on tissue microculture plates (Limbro Chemical Company, New Haven, CT, USA). The cells were stimulated separately with 10 µg/ml of leishmania antigen, 1 µg/ml of PPD (Connaught Laboratories, Ontario, Canada), 5 LF/ml of TT antigen (Wyeth-Ayerst Lab., Marietta, PA, USA), 25 µg/ml of *C. albicans* antigen, and 25 ng/ml of mAb αCD3. After 3 and 5 days of incubation (37ºC, 5% $CO_2$), 1 µCi of [$^3$H]-thymidine (6.7 Ci/mmol; New England Nuclear, Boston, MA, USA) was added to the cultures for 5 h. The cultures were then harvested and processed, and [$^3$H]-thymidine incorporation was measured with a scintillation counter.

The results are reported as the means of the cpm of triplicate cultures ± SEM and as percent suppression. Statistical analysis was performed by the Wilcoxon signed rank test.

### Monoclonal antibodies

The mAb W6/32 (IgG2a) directed at a

framework determinant of HLA class I molecules was used at a concentration of 25 µg/ml. This antibody recognizes a framework epitope expressed on virtually all HLA class I molecules which has been conserved throughout evolution since it is expressed in a large number of primates (6). The mAb L2.43 directed at a monomorphic determinant of HLA class II molecules was utilized also at a concentration of 25 µg/ml. This antibody was obtained from the American Tissue Culture Collection and is an anti-HLA-DR. It was purified from ascitic fluid by the caprylic acid precipitation method (7).

### Production and determination of IFN-γ *in vitro*

IFN-γ levels were determined in supernatants of stimulated cell cultures. Briefly, PBMC (3 x $10^6$) were stimulated with leishmania antigen at a concentration of 20 µg/ml in the presence or absence of the anti-HLA class I mAb W6/32 at a concentration of 25 µg/ml. After 3 days of incubation at 37°C and 5% $CO_2$, the supernatants were collected and the levels of IFN-γ were determined by ELISA using a sandwich technique. The results are reported as pg/ml.

### Evaluation of the addition of mAb W6/32 to pulsed macrophages in terms of the inhibition of T cell proliferation

For these experiments, PBMC were adjusted to a concentration of $10^6$/ml RMPI 1640 medium supplemented with 10% AB heat-inactivated sera, and aliquots of 0.2 ml were added to microtiter plate wells and incubated for 2 h at 37°C. Non-adherent cells were then removed, and adherent cells were incubated with antigen (25 µg/ml) for about 2 h. The antigen was then washed and the W6/32 mAb (25 µg/ml) was added. After 2 h, the wells were gently washed and the non-adherent cells were added. Alternatively, adherent cells were first incubated with the antigen for 2 h and washed and non-adherent cells and W6/32 mAb were then added simultaneously.

Cultures of adherent and non-adherent cells plus antigen in the absence of the inhibiting mAb were used as positive control. The blastogenesis assay was performed as previously described.

## Results

The effect of mAb to MHC I and II molecules on the lymphocyte proliferative response to leishmania antigen in patients with tegumentary leishmaniasis is shown in Figure 1. Although the magnitude of the leishmania-specific response varied, a significant response was observed in all the individuals tested. In six patients (MS, MP, AO, CO, AC and CS) addition of a mAb to MHC class I molecules had no significant effect on the lymphocyte proliferative response. In the remaining five patients the anti-MHC I mAb W6/32 suppressed the response to leishmania antigen by 42 to 67% ($P<0.01$) (Figure 1). The suppression was greater in patients with a relatively low response to leishmania antigen. In contrast, the anti-HLA class II mAb L2.43 had a more homogeneous inhibitory effect on lymphocyte proliferation, suppressing the response

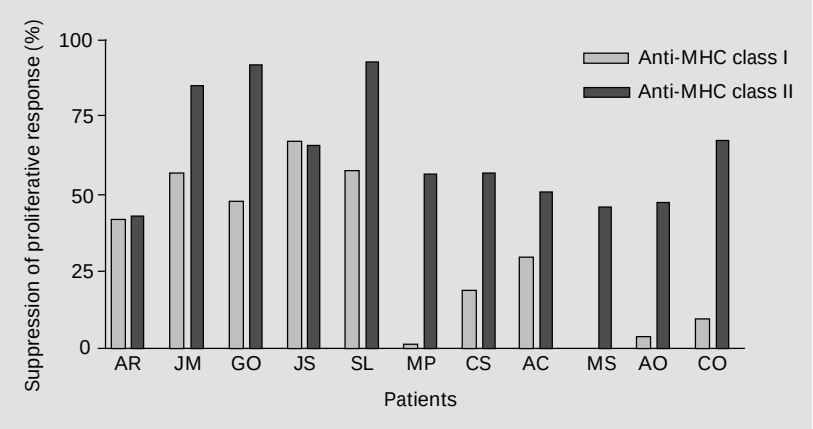


Figure 1 - Effect of anti-MHC class I and II mAbs on the lymphocyte proliferative response to leishmania antigens in patients with tegumentary leishmaniasis.

by 43% or more in all patients studied ($P<0.01$) (Figure 1).

In contrast to the variability of the suppression of leishmania responses, the mAb W6/32 consistently suppressed (≥95%) T cell proliferation to stimulation induced by αCD3. Mean [$^3$H]-thymidine uptake in αCD3-stimulated cultures in the absence and presence of the mAb W6/32 was 70366 ± 11599 and 3428 ± 1988, respectively. In addition to lymphocyte proliferation, IFN-γ production in response to leishmania antigen was also suppressed by the anti-HLA class I mAb (Figure 2). The suppression was variable: in three patients IFN-γ levels were suppressed by more than 60%, while in the other two IFN-γ levels were only 26 and 10% lower than those observed in cultures stimulated with antigen alone.

The ability of the anti-HLA class I mAb to suppress the proliferative response to PPD, *C. albicans* and TT antigens in tegumentary leishmaniasis patients (Table 1) and in healthy subjects was also examined. The magnitude of the suppression observed was not dependent on the antigen or on the patients. For instance, as shown in Table 1, the reduction of the response to PPD varied, ranging from 64% (patient SL) to 6% (patient JS). Similarly, a decrease in the responses from the same donor stimulated with different antigens was quite variable. Thus, in patient SL, mAb W6/32 reduced the proliferative response to TT only by 25%, but the same antibody suppressed the response to PPD by 64%.

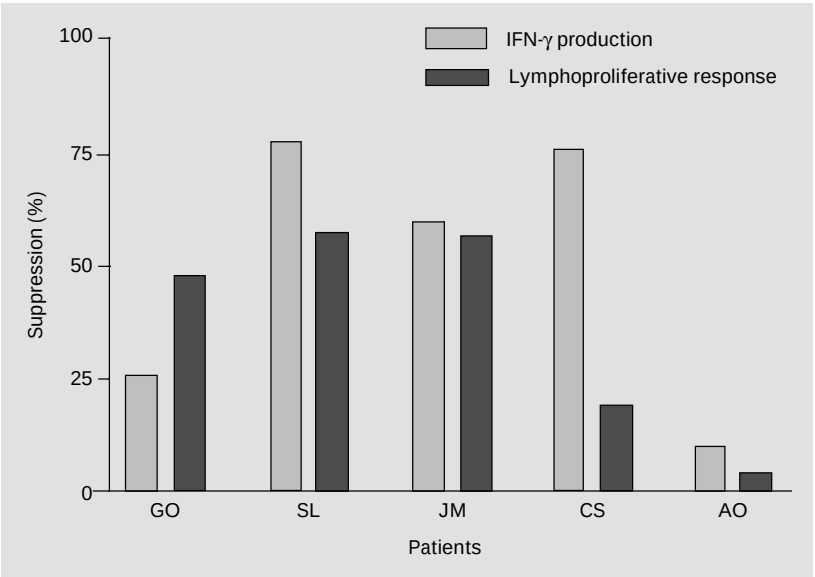


Figure 2 - Effect of anti-MHC class I mAb on IFN-γ synthesis and lymphoproliferative response in patients with tegumentary leishmaniasis.

The effect of W6/32 on the lymphoproliferative response of six healthy subjects to PPD, *C. albicans* and TT was also tested. In these experiments, the magnitude of the suppression mediated by the HLA I mAb varied with the antigens and donors tested. The suppression of the response to PPD, *C. albicans* and TT in donor AJ was 88, 62 and 57%, respectively. However, in cultures from donor DP stimulated with the same antigens, mAb W6/32 reduced the response by 22, 20 and 8%, respectively. There was also a variability in the magnitude of the suppression of the responses to different antigens within the same individual. In donor EC, the response to *C. albicans* was reduced by 60% while the response to TT was inhibited by 6%. Similarly to the results obtained for tegumentary leishmaniasis patients, the suppression mediated by W6/32 in healthy donors was somehow related to the degree of the response to the antigen tested.

In order to assess whether the inhibitory effect of the anti-HLA I mAb is primarily directed at antigen presenting cells (APCs) or at the responding T cells, experiments with antigen-primed non-adherent cells, separately incubated with W6/32, were performed. As shown in Table 2, when APCs were first pulsed with leishmania antigens in the presence of mAb W6/32, no inhibitory effect was observed. In contrast, when mAb W6/32 was added after the addition of lymphocytes to the cultures, suppression of proliferation was observed. Similarly, in PBMC from healthy control subjects stimulated with *C. albicans* antigen, the suppression mediated by mAb W6/32 was observed only when

the mAb was added to cultures of Ag-pulsed APCs and T cells. In contrast, anti-HLA class II mAbs suppressed the lymphoproliferative response in both conditions, when added first to Ag-pulsed APCs alone or to Ag-pulsed APCs and T cells.

## Discussion

The ability of monoclonal antibody to HLA determinants to suppress lymphocyte proliferation induced by αCD3 (6), by the mitogen phytohemagglutinin (PHA) (8) and by autologous cells (1) has been previously demonstrated. Soluble antigens are presented in the context of HLA class II molecules and, as expected, monoclonal antibodies against HLA class II suppress lymphocyte proliferation mediated by such antigens. The present study provides evidence that a mAb directed at an HLA class I framework determinant is capable of suppressing lymphocyte proliferation and cytokine secretion in response to several soluble antigens. These observations were first documented in the present study in patients with tegumentary leishmaniasis and later in lymphocyte cultures from healthy subjects stimulated with PPD, TT and *C. albicans*.

Table 1 - Effect of anti-MHC I mAb on T cell responses to PPD, *C. albicans* and tetanus toxoid antigens in patients with tegumentary leishmaniasis.

Data are reported as mean [$^3$H] uptake in cpm ± SD. The numbers in parentheses indicate the extent of suppression by mAb W6/32 at 25 µg/ml. ND, Not determined.

| Culture conditions | Patients | | | |
|---|---|---|---|---|
| | JS | SL | CS | AC |
| PPD + medium | 7016 ± 496 | 20431 ± 2110 | 18222 ± 1628 | ND |
| PPD + W6/32 | 6599 ± 1108 (6%) | 7553 ± 1467 (64%) | 12725 ± 2325 (31%) | ND |
| *C. albicans* + medium | 56363 ± 728 | 31558 ± 4790 | 4072 ± 732 | 21942 ± 5115 |
| *C. albicans* + W6/32 | 36363 ± 3568 (35%) | 20714 ± 1023 (35%) | 1815 ± 752 (56%) | 18535 ± 3245 (16%) |
| T. toxoid + medium | 17452 ± 4805 | 36291 ± 3551 | ND | 9708 ± 2429 |
| T. toxoid + W6/32 | 16132 ± 2840 (8%) | 27305 ± 1859 (25%) | ND | 6372 ± 1165 (35%) |
| Leishmania Ag + medium | 76356 ± 1284 | 39325 ± 2533 | 63947 ± 5035 | 112974 ± 3013 |
| Leishmania Ag + W6/32 | 22988 ± 1084 (67%) | 16744 ± 1400 (58%) | 52030 ± 1411 (19%) | 80000 ± 15896 (30%) |

Table 2 - Effect of the addition of anti-HLA class I and II mAbs to cell populations on the response to leishmania.

*Anti-HLA class II mAb; **anti-HLA class I mAb. The numbers in parentheses indicate the extent of suppression by the mAb.

| Clinical conditions | Culture conditions | | | | |
|---|---|---|---|---|---|
| | Adherent cells + Ag Non-adherent cells Medium | Adherent cells + Ag mAb L243* Non-adherent cells | Adherent cells + Ag Non-adherent cells mAb L243 | Adherent cells + Ag mAb W6/32** Non-adherent cells | Adherent cells + Ag Non-adherent cells mAb W6/32 |
| Tegumentary leishmaniasis | 67745 ± 5006 | 47470 ± 4866 (30%) | 11939 ± 3084 (82%) | 71576 ± 7879 (0%) | 24453 ± 2244 (64%) |
| Tegumentary leishmaniasis | 30878 ± 4124 | 15527 ± 1964 (50%) | 2074 ± 489 (93%) | 29110 ± 8156 (6%) | 12014 ± 728 (61%) |
| Tegumentary leishmaniasis | 155363 ± 22841 | 85643 ± 8485 (45%) | 57662 ± 5929 (63%) | 186582 ± 6875 (0%) | 178400 ± 86947 (0%) |
| Healthy subject (*C. albicans* Ag) | 19634 ± 100 | 11736 ± 1294 (40%) | 2710 ± 766 (86%) | 28066 ± 533 (0%) | 893 ± 188 (96%) |

In patients with tegumentary leishmaniasis, the suppression of antigen-specific lymphoproliferative T cell responses mediated by an anti-HLA class I mAb was variable and demonstrable in only half of the patients tested. Individual variation in the magnitude of the inhibition of the proliferative response has been observed by others when PHA-P was used as a stimulus (9). The same variability in the extent of suppression was observed when PBMC from these patients and from healthy donors were stimulated with PPD, *C. albicans* and TT.

Anti-HLA class I mAb may have costimulatory or down-regulatory effects on T cell proliferative responses induced through distinct activation pathways. Thus, while mAbs to distinct monomorphic determinants of HLA class I molecules have been shown to enhance CD2-induced T cell proliferation in 30% of the PBMC populations tested (10), the same mAbs exert inhibitory regulation on anti-CD3- (6) or mitogen- (8) induced T cell proliferation. This indicates that the same HLA class I determinants play a differential regulatory role in T cell activation induced via different pathways. Furthermore, it has been shown that both inhibition and activation can be regulated by the degree of aggregation of membrane-bound HLA class I molecules. Thus, while crosslinking of mAb specific for polymorphic HLA-A and HLA-B, or monomorphic determinants increases their inhibition of PHA-induced proliferation, aggregation of HLA class I molecules enhances proliferation of PBMC in the presence of sub-mitogenic doses of polymorphonuclear antigens (PMA) (11). Therefore, it is possible that the degree of HLA class I aggregation and the use of different T cell activation pathways may account for the observed individual variation in the suppression mediated by anti-HLA class I mAbs.

In experiments carried out to determine the target cell population for the suppression mediated by anti-HLA class I mAb, inhibition of proliferative response was only demonstrated when the mAb was added in the presence of lymphocytes. The possibility that anti-HLA I mAb is present in oversaturating concentrations throughout the culture period so that suppression can be observed is unlikely, since mAb W6/32 has relatively high affinity and a slow dissociation rate (6). This may reflect the direct interaction of anti-HLA class I mAb with T cells (10). Although Fc receptors expressed by monocytes play an important role in the regulation of T cell responses to anti-CD3 antibodies (12-14), inhibition of T cell proliferation by anti-HLA class I mAb does not require Fc-receptor crosslinking (8). Similarly, the suppression of PHA-P-induced T cell proliferation mediated by mAb to HLA class I is monocyte independent (9). In contrast, when anti-HLA class II mAbs were used, suppression was predominantly observed when antibodies were incubated with non-adherent cells. The inhibition may be mediated by blockage of transcriptional activation of genes leading to IL-2 and IL-2R gene expression, and at least in part, by down-regulation of IFN-$\gamma$ production.

Recently, we have identified a novel regulatory CD8+ T cell subset which lacks the expression of CD28 molecules, secretes IL-10 and is expanded with aging (15). Interestingly, this CD8+ T cell subset expresses NK inhibitor receptors (KIR) which are specific for polymorphic HLA I determinants that mediate inactivation of NK (16,17) and T cells (18). Thus, in our leishmaniasis patients, blockade of HLA I by mAbs may prevent inactivation of KIR+ regulatory T or NK cells. These cells, in turn, will produce IL-10, a cytokine involved in immunosuppression in leishmaniasis (19). Alternatively, it has been shown that enhancement of responses of KIR+T cells occurs by way of NK receptor co-stimulation. In this case, blocking HLA-I molecules will prevent the co-stimulatory enhancement. Whether KIR+ regulatory T cell subsets are present in leish-

maniasis patients remains to be determined.

In the present study we have shown that MHC class I was able to suppress lymphocyte proliferation and IFN-γ production. The effect of MHC mAb was similar in three cases but in two cases there was a marked difference in the extent of suppression of these two functions. These differences can be explained by the fact that these functions are not necessarily performed by the same cell (20).

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# Announcement

## 1999 Award in honor of Fred L. Soper (1893-1976) for publications in the field of Inter-American Health

This is an announcement and call for submission of nominations for the 1999 award in honor of Fred L. Soper, former Director of the Pan American Health Organization (the World Health Organization Regional Office for the Americas) from 1947 to 1958.

In addition to his service with PAHO/WHO, Dr. Soper played a major role in the fight against yellow fever and other infectious diseases in Brazil as part of his work with the Rockefeller Foundation in the 1920s and 1930s and in the control of typhus in North Africa and Italy during the Second World War. He was one of the truly major figures of the century in inter-American health.

The Award is presented annually to the author or authors of an original scientific contribution comprising new information on, or new insights into, the broad field of public health, with special relevance to Latin America or the Caribbean or both. This may consist of a report, an analysis of new data, experimental or observational, or a new approach to analyzing available data. Preference is given to studies involving more than one discipline and to papers related to infectious disease, a life-long concern of Dr. Soper.

Only papers already published in scientific journals listed in the Index Medicus or in the official journals of the Pan American Health Organization are eligible for consideration. Furthermore, the Award is limited to contributions by authors whose principal affiliation is with teaching, research or service institutions located in the countries of Latin America and the Caribbean (including the Centers of the Pan American Health Organization).

The Award Fund is administered by the Pan American Health and Education Foundation (PAHEF), which receives voluntary contributions designated for the purpose and holds them in a separate fund. The Award consists of a suitable certificate and a monetary prize of US$1000.00. The winner(s) of the Award each year is nominated by an Award Committee, composed of representatives designated by PAHO and by PAHEF; final selection is made by the Board of Trustees of PAHEF.

Papers submitted by or on behalf of their authors may be considered for the Fred L. Soper Award. For purposes of the 1999 Award, only papers published during calendar year 1998 will be considered; all submissions must be received by 31 March 1999 at the following address:

**Executive Secretary**
**PAHEF**
**525 23rd Street N.W.**
**Washington, DC 20037**
**USA**
**e-mail: marksric@paho.org**