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Immunology

Effect of anti-HLA antisera on macrophage–T-cell interaction

(antigen-induced proliferation/adherent accessory cells/DRw antigens/human histocompatibility restriction)

RAIF S. GEHA*, HENRY MILGROM*†, MARTIN BROFF*, SUSAN ALPERT*, SHARON MARTIN‡, AND EDMOND J. YUNIS‡

*The Divisions of Allergy and Immunology, Department of Medicine, The Children's Hospital Medical Center and Harvard Medical School, Boston, Massachussetts 02115; †Scripps Clinic and Research Foundation, 10666 North Torrey Pines Road, La Jolla, California 92037; and ‡Sidney Farber Cancer Research Institute, Boston, Massachusetts 02115

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ABSTRACT **Human T lymphocytes were shown to proliferate in response to tetanus toxoid antigen only in the presence of macrophages. This response was inhibited by anti-DRw but not by anti-HLA (A and B loci) antisera added to the cultures and by pretreatment of macrophages but not of T cells with anti-DRw antisera and complement. Macrophages pulsed for 18 hr with antigen and then washed were capable of triggering T-cell proliferation. Addition of anti-DRw but not anti-HLA (A and B loci) antisera during the pulse period inhibited the macrophages' ability to trigger T-cell proliferation. The data obtained indicate that human T cells recognize and proliferate in response to antigen presented by the macrophages in association with Ia-like antigens.**

It has been shown in experimental animals that T lymphocytes respond to antigens only in the presence of macrophage-like accessory cells and that native antigen alone is insufficient to cause T-cell activation (1). In addition, many recent studies have shown that T-cell recognition of antigen presented by accessory cells involves recognition of major histocompatibility complex antigens—namely, the Ia antigens in the guinea pig and the IA and IE/IC subantigens in the mouse (2, 3).

The present studies were designed to study human macrophage–T-cell interaction in the proliferative response to the soluble antigen, tetanus toxoid (TT). The data presented indicate that this response has an absolute requirement for macrophage (Mϕ) and is inhibited by anti-DRw but not anti-HLA antisera and that the inhibitory effect of anti-DRw antisera is exerted at the level of the Mϕ.

MATERIALS AND METHODS

Antigen. TT was obtained from the Massachusetts Biological Laboratories (Lot LP430PM) and dialyzed extensively against 0.15 M NaCl.

Antisera and Tissue Typing. Anti-HLA antisera (A, B, C, and DRw loci) were obtained from multiparous women whose informed consent was obtained. The antibody specificity of the antisera was determined by testing against panels of typed lymphocytes (4). These antisera were cytotoxic to lymphocytes at a final dilution of 1:4 in the microcytotoxicity assay. The anti-HLA-A,B antisera used in the cell culture experiments were shown to have no DRw reactivity, because after exhaustive absorption (three cycles) with pooled platelets they showed no reactivity with a panel of purified B lymphocytes. HLA-ABC activity was removed from anti-HLA DRw antisera by absorption with random pools of platelets (5). Anti-DRw antisera were cytotoxic to B cells at a final dilution of 1:4 in a microcytotoxicity assay.

HLA typing was performed on Ficoll/Hypaque isolated lymphocytes by microcytotoxicity assay as described (4). DRw typing was performed on B lymphocyte-rich suspensions as described in ref. 4.

Preparation of T Cells and Macrophages (Mϕ). Mononuclear cells were isolated from healthy adult donors over Ficoll/Hypaque (Pharmacia), washed three times with Hanks' balanced salt solution containing 1% heat-inactivated AB^+ serum, and suspended at 3×10^6 cells per ml in medium RPMI 1640 (Microbiological Associates, Walkersville, MD) containing glutamine (2 mM), penicillin (100 units/ml), streptomycin (50 μg/ml), and 20% AB^+ serum. Cell suspensions were incubated for 2 hr in 100 × 15 mm petri dishes (Falcon) at 10 ml per dish at 37°C in 95% air/5% CO_2. Nonadherent cells were collected by three vigorous rinses with Hanks' solution and filtered through nylon wool (Fenwal Laboratories, Deerfield, IL) to remove remaining adherent cells and B cells as described by Julius *et al.* (5). These preparations served as a source of T cells. Their characteristics are given in Table 1. Adherent cells were recovered from the petri dishes by incubating the dishes for 15 min in phosphate-buffered saline (pH 7.2) at 4°C. The adherent cells were then scraped off with a rubber policeman, washed two times in Hanks' solution, resuspended in culture medium, and served as a source of Mϕ. Their characteristics are given in Table 1.

Antigen-Induced T-Cell Proliferation. Cultures were carried out in triplicate in microtiter plates (Linbro) in 0.2 ml of complete culture medium consisting of RPMI 1640 supplemented with 10% heat-inactivated AB^+ serum. Standard cultures contained 1×10^5 unfractionated cells, 1×10^5 T cells with or without the addition of 1×10^4 macrophages, or 1×10^4 macrophages. TT was added to antigen-stimulated cultures in 20 μl of RPMI 1640 at a final concentration of 10 μg/ml. Incubation was carried out for 6 days at 37°C in a humidified atmosphere of 95% air/5% CO_2. The cultures were then pulsed overnight with 0.8 μCi (1 Ci = 3.7×10^{10} becquerels) of [*methyl*-^{3}H]thymidine (New England Nuclear) in 20 μl of RPMI 1640 and were harvested in a multiple sample harvester (Skatron, A. S., Lierbyen, Norway). The radioactivity was assayed in a Packard Tri-Carb liquid scintillation spectrometer (Packard Co., Downers Grove, IL).

Effect of Antisera to HLA (A and B loci) and DRw Antigens on Mϕ–T Cell Interaction. (*i*) *Pretreatment of cells with anti DRw antisera with or without complement.* Cells were exposed to anti DRw antisera (5×10^6 cells per ml in complete culture medium containing a 1:5 dilution of antiserum) for 30 min on ice. After a single wash the cells were resuspended in 1 ml of RPMI 1640 medium containing a 1:10 dilution of fresh frozen pooled rabbit serum as a source of complement. This

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Abbreviations: B cells, bone marrow-derived lymphocytes; Mϕ, macrophages; T cells, thymus-derived lymphocytes; TT, tetanus toxoid.

Table 1. Characteristics of T cell-rich and Mϕ-rich populations

Cell population	Viability, %	Cells ingesting latex, %	Cells staining for peroxidase, %	Cells forming E rosettes, %	Cells forming EAC3 rosettes, %	Cells with membrane sIg, %
T cell-rich	>95	<1	<1	94 ± 3	<2	<1
Mϕ-rich	>90	78 ± 11	85 ± 8	<1	88 ± 6	<2

Values represent mean ± SD of four different experiments. E, Sheep erythrocytes; EAC3, EAC 1423; sIg, surface immunoglobulins. Staining for surface immunoglobulin was performed by using the $F(ab')_2$ IgG fragment of a fluorescein-conjugated rabbit anti-human immunoglobulin (polyvalent) antiserum.

rabbit serum was preabsorbed with pooled platelets and had low toxicity for human mononuclear and adherent cells (less than 5% killing). After an incubation period of 1 hr at 37°C, the cells were washed three times in Hanks' solution, assessed for viability by trypan blue exclusion, counted and resuspended in complete culture medium, and cultured at a final concentration of 10% viable Mϕ. In some experiments the complement treatment was omitted and the cells were washed three times prior to culture.

(ii) Effect of the addition of antisera to HLA (A and B loci) and DRw antigens to cell cultures. Antigen-stimulation of cultures containing 90% T cells and 10% Mϕ was carried in the presence of 10% human AB^+ serum or in the presence of 10% anti-HLA- or anti-DRw-containing human serum.

(iii) Effect of antisera on the ability of antigen-pulsed Mϕ to trigger T-cell proliferation. The optimal conditions of pulsing macrophages with antigen were as follows: Macrophages suspended in complete culture medium were incubated in petri dishes with TT antigen (10 μg/ml) for 18 hr at 37°C after which the dishes were washed extensively with complete culture medium. Mϕ were recovered from the plates as described above and added to T cells at a final concentration of 10%. The amount of TT antigen remaining associated with Mϕ was determined by pulsing Mϕ with 10 μg of TT antigen per ml to which a trace amount of ^{125}I-labeled TT [radiolabeled by the Chloramine-T method (6)] was added. Under the present conditions of antigen pulsing, less than 1 ng of TT antigen remained associated with 1×10^4 Mϕ used in each culture. This would correspond to $30–50 \times 10^4$ molecules per Mϕ assuming all the radiolabel remained associated with intact TT molecules. In some experiments pulsing with antigen was performed in the presence of RPMI 1640 medium supplemented with 10% human serum containing anti-HLA or anti-DRw antibodies.

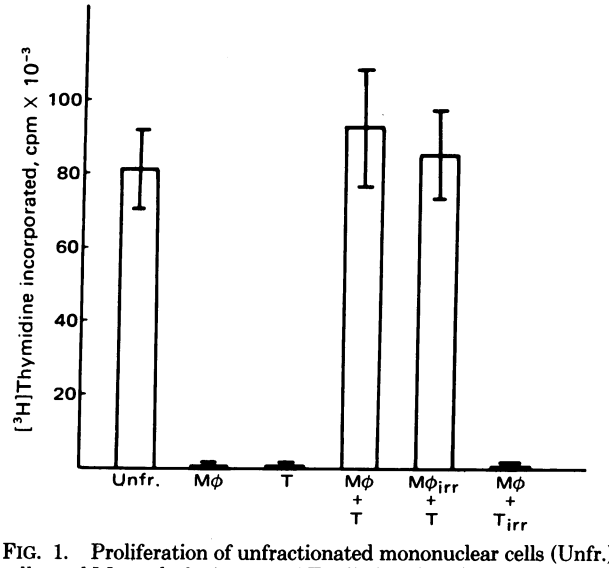

FIG. 1. Proliferation of unfractionated mononuclear cells (Unfr.), T cells, and Mϕ and of mixtures of T cells (90%) and Mϕ (10%) in response to TT antigen. Irradiated (irr) cell populations received 5000 rads. Results represent the pooled data (average ± SD) of six experiments expressed as cpm of [^{3}H]thymidine incorporated per culture.

RESULTS

Requirement of Mϕ for Antigen-Induced T-Cell Proliferation. Purified populations of T cells and of Mϕ failed to proliferate in response to TT antigen. Addition of 10% Mϕ to T cells resulted in a proliferative response comparable to that of unfractionated mononuclear cells. Irradiation experiments established that the proliferating cell in this mixture was the T cell and that the accessory function of the Mϕ is resistant to irradiation (Fig. 1).

Effect of Pretreatment of T Cells and Mϕ with Antisera to DRw Antigens. Treatment of Mϕ with anti-DRw antisera and complement resulted in lysis of 60–80% of the cells as determined by trypan blue exclusion. The Mϕ that survived this treatment were greatly diminished in their capacity to support T-cell proliferation to antigen. Treatment of T cells with anti-DRw antisera and complement resulted in the lysis of less than 10% of the cells and did not affect the ability of the T cells to proliferate in response to antigen in the presence of untreated Mϕ (Table 2). Pretreatment of Mϕ or T cells with anti-DRw antisera without the addition of complement did not interfere with the ability of T cell–Mϕ mixtures to proliferate in response to antigen.

Table 2. Effect of pretreatment of Mϕ and T cells with anti-DRw antisera and complement (C) on the proliferative response of a DRw4/DRw5 (DRw4,5) donor to TT antigen*

Cells pretreated	Pretreatment	Cultures stimulated with	[^{3}H]Thymidine incorporated per culture, cpm
Mϕ	Medium	Medium	585 ± 137
	Medium	TT	78,350 ± 3,763
	Anti-DRw4,5 + C	TT	16,531 ± 1,140
	Anti-DRw4,5	TT	69,862 ± 5,428
	Anti-DRw3,7 + C	TT	64,608 ± 6,360
	Anti-DRw3,7	TT	74,274 ± 5,079
	C	TT	70,326 ± 3,078
T	Medium	Medium	726 ± 109
	Medium	TT	71,453 ± 2,621
	Anti-DRw4,5 + C	TT	66,483 ± 2,380
	Anti-DRw4,5	TT	69,177 ± 1,942
	Anti-DRw3,7 + C	TT	68,500 ± 3,188
	Anti-DRw3,7	TT	72,985 ± 3,357
	C	TT	67,309 ± 4,116

* The HLA phenotype of the donor was HLA-A3, AW32, B7, B14, DRw4, DRw5.

† Similar results were obtained in four different experiments.

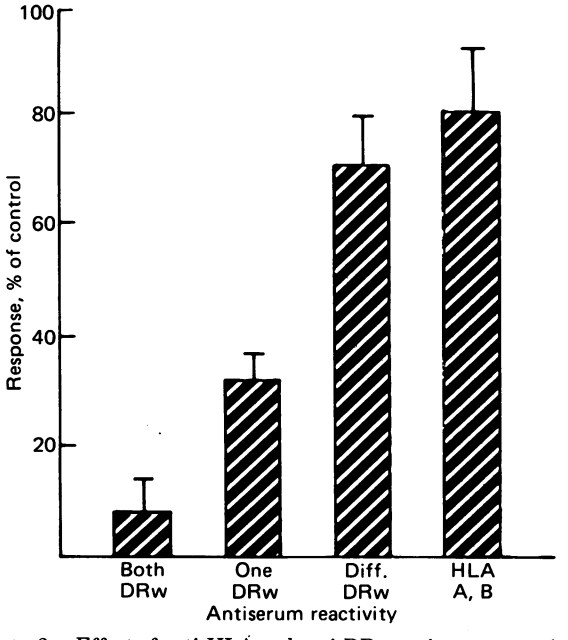

FIG. 2. Effect of anti-HLA and anti-DRw antisera present in the culture medium on the proliferative response of mixtures of T cells (90%) and Mϕ (10%) cultured for 6 days in the presence of TT (10 μg/ml). Results are expressed as percentage (±SD) of the value for control cultures. Antisera were directed against both DRw antigens of the cell donor, one of the two DRw antigens of the cell donor, DRw antigens different from those of the cell donor, and HLA antigens of the cell donors. Five donors were used in this experiment. (S.A.: HLA-A3, W32, B7, B14, DRw4, DRw5; D.T.: HLA-A11, AW31, B15, BW51, DRw3, DRw5; R.G.: HLA-A2, A11, BW35, BW4, DRw5, DRw6; J.M.: HLA-A3, AW30, B7, B13, DRw2, DRw7; B.W.: HLA-A1, A3, B8, B18, DRw3, DRw5.) The anti-DRw antisera used in this experiment were the following: Third American HLA Workshop numbers A57, A33, D17, B04, and D24 recognizing, respectively, the following specificities: DRw2; DRw3; DRw4, DRw5, and DRw6; DRw3 and DRw6; and DRw3; Seventh International Histocompatibility Workshop numbers 7W152, 7W010, and 7W149 recognizing, respectively, specificities DRw1, DRw1 and DRw2, and DRw7; and four antisera from our laboratory submitted to the Eighth International Workshop (designation Ia 279, Ia 280 and Ia 193, and Ia 16) recognizing, respectively, DRwH; DRw4 plus DRw7, and DRw4 plus DRw5 specificities. The HLA (A and B loci) antisera used, devoid of DRw reactivity, were the following: SFCI 51 recognizing HLA-A2, SFCI 63 recognizing HLA-B7, RC 49 recognizing HLA-A3, RC 74 recognizing HLA-B7, and RC 171 recognizing BW51.

Effect of the Continuous Presence of Antisera to HLA (A and B Loci) or DRw Antigens on the Proliferative Response of T Cell/Mϕ Mixtures to TT. As shown in Fig. 2, the Mϕ-dependent T-cell proliferative response to TT was inhibited by the continuous presence of antisera to DRw antigens in the cultures ($P < 0.01$). It was noted that antisera containing reactivity against two DRw specificities were more inhibitory to cells of donors containing both of these specificities than to cells of donors containing only one of these specificities ($P < 0.05$). In contrast, sera containing anti-HLA antibodies (A and B loci) reactive with the lymphocytes of the cell donor, as well as sera containing anti-DRw antisera not reactive with the donor antigen, did not produce significant inhibition of the antigen-proliferative response when added to the cell cultures for the whole duration of the culture period.

Mϕ Pulsed with Antigen in the Presence of Anti-DRw Antisera Do Not Trigger T-Cell Proliferation. Mϕ pulsed for 18 hr with TT caused T cells to proliferate to a degree comparable to that seen when T cells were incubated in the presence

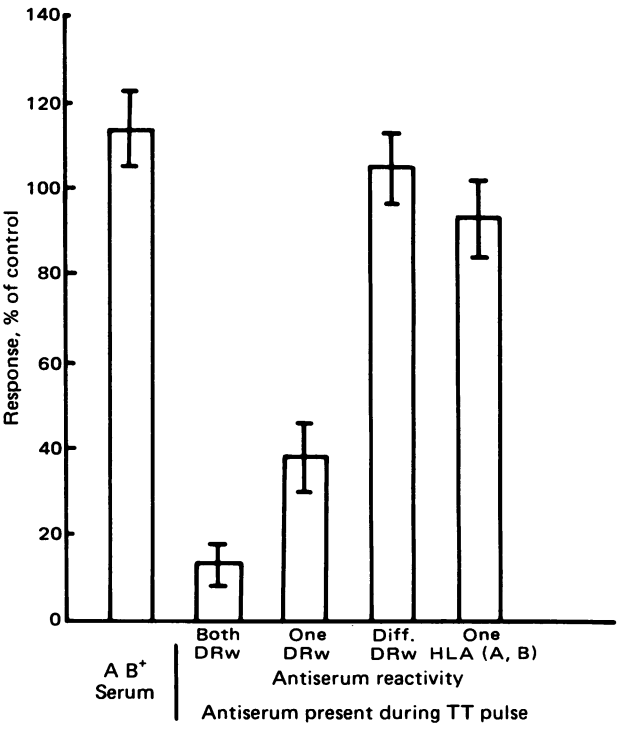

FIG. 3. Effect of antisera to major histocompatibility complex antigens on the ability of antigen-pulsed Mϕ to trigger T cells. Antisera and TT antigen were added simultaneously to Mϕ. After an 18-hr incubation period, the Mϕ were vigorously washed and then added at a final concentration of 10% viable Mϕ to T cells. After 6 days, DNA synthesis was assayed and compared to control cultures containing 90% T cells, 10% Mϕ, and soluble TT antigen (10 μg/ml). Results are expressed as percentage (± SD) of the control cultures. Cells from the same five donors and the same anti-HLA (A, B, and DRw loci) antisera were used in this experiment, as described in the legend of Fig. 2.

of Mϕ and soluble TT. Mϕ pulsed with TT in the presence of antisera recognizing both DRw specificities of the cell donor failed to trigger T-cell proliferation (Fig. 3). Mϕ pulsed with TT in the presence of antisera recognizing only one of the two DRw antigens of the cell donor were inhibited, but to a lesser extent ($P < 0.05$), in their ability to trigger T-cell proliferation. Addition during the pulse period of antisera to HLA antigens of the cell donor or addition of antisera to DRw antigens not present on the donor cells did not significantly affect the ability of the Mϕ to trigger T-cell proliferation.

DISCUSSION

The present experiments demonstrate an absolute requirement for accessory adherent cells in the T-cell proliferative response to soluble antigen. This function of the adherent cells is radioresistant. The adherent cells used in our experiments contained from 67 to 89% phagocytic cells capable of ingesting latex. Therefore, it is not clear from the present experiments whether the accessory cell necessary for T-cell proliferation in response to antigen is a classical Mϕ or an adherent nonphagocytic null cell as suggested by Couring *et al.* in the mouse system (3).

The adherent cell required for T-cell proliferation was found to bear DRw antigens similar to those present on the B lymphocytes of the cell donor. Treatment of adherent cells with anti-DRw antisera and complement resulted in the lysis of a significant number of these cells (60–80%) and in the inability of the adherent cells surviving this treatment to support antigen-induced T-cell proliferation. In contrast, similar treatment of T cell-rich populations did not affect the T-cell proliferative

response to antigen, indicating that under the conditions of the present experiments the resting T cell that subsequently proliferates in response to antigen stimulation does not belong to the recently described subset of resting T cells bearing DRw antigens (7).

The Mϕ-dependent T-cell proliferative response to TT antigen was inhibited by antisera to DRw antigens but not by antisera to HLA antigens of the cell donor (Fig. 2). Antisera to both DRw antigens of the cell donor were very strongly inhibitory, whereas antisera that recognized only one of the two DRw antigens were only moderately inhibitory to the T-cell proliferative response.

These results demonstrate that anti-DRw antisera interfere with Mϕ–T cell interaction, but do not determine whether the inhibitory effect of the anti-DRw antisera is exerted at the level of the Mϕ, at that of the T cell, or at that of both Mϕ and T cell, because it has been shown that proliferating human T lymphocytes synthetize and express on their surface Ia determinants (8). In the guinea pig it has been shown that the inhibitory effect of anti-Ia antisera is exerted at the level of the macrophage. Indeed, strain 13 T cells depleted of alloreactivity against strain 2 Mϕ were not able to proliferate in response to antigen presented by strain 2 Mϕ in the presence of anti-Ia antisera reactive with strain 2 Ia antigens. In contrast, antisera to Ia antigens of strain 13 did not interfere with this response (9).

Because it was found that human Mϕ pulsed with antigen effectively trigger T-cell proliferation, the effect of anti-DRw antisera on antigen pulsing of Mϕ was studied to determine whether the Mϕ is the target of the inhibition seen in our experiments by anti-DRw antisera. These experiments, in which Mϕ from DRw heterozygous individuals were utilized, demonstrated that the presence of anti-DRw antisera during the 18-hr antigen pulse period interfered with the ability of the pulsed Mϕ to trigger T-cell proliferation. Sera with specificities to both DRw antigens of the cell donor resulted in complete inhibition of the ability of the antigen-pulsed Mϕ to trigger T-cell proliferation. Monospecific sera or duospecific sera reactive with only one of the DRw antigens of the cell donor resulted in an intermediate level of inhibition. These findings indicate that DRw determinants are expressed separately on the macrophages of heterozygous individuals and suggest that in man, like in the mouse and the guinea pig, Ia antigens are necessary for the formation of immunogenic complexes with antigen that can be recognized by T cells.

A trivial explanation for our findings would be that anti-DRw antisera prevent antigen uptake by the Mϕ. Such an explanation was ruled out by studies using ^{125}I-labeled TT, which showed that as much antigen was taken up by Mϕ in the presence of anti-DRw antisera as in the absence of such antisera. Also, because pretreatment of Mϕ for 18 hr with anti-DRw antisera did not interfere with the ability of the Mϕ to trigger T cells in the presence of soluble antigen (Table 2), it is safe to presume that treatment of Mϕ with anti-DRw antisera does not interfere with subsequent resynthesis and reexpression of Ia antigen. Henceforth, the failure of Mϕ pulsed with antigen in the presence of anti-DRw antisera to trigger T-cell proliferation suggests that Mϕ-processed antigen may not remain indefinitely available to associate with Ia-like antigens to form immunogenic complexes that can be recognized by T cells.

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