

Ia DETERMINANTS ON STIMULATED HUMAN T LYMPHOCYTES

Occurrence on Mitogen- and Antigen-Activated T Cells*

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Considerable interest has been focused on a group of human alloantigens expressed primarily on B lymphocytes which closely resemble the Ia antigens delineated in the mouse (1-3). These Ia-like antigens, hereafter referred to as Ia, exist as a two-chain bimolecular complex of $\cong 28,000$ and $37,000$ daltons. Apart from B cells, they have been found on the surfaces of monocytes, leukemic blasts, Langerhans cells, endothelial cells, spermatozoa, and precursors of both erythroid and myeloid series. The occurrence of these antigens on T cells has been a controversial topic in both the human and murine systems (4-6). More recently, clear evidence for the occurrence of I-J antigens on suppressor T cells and on certain suppressor factors has been obtained in the mouse (7, 8). In the human, a small but definite number of E-rosette-positive T cells have been shown to carry Ia antigens (9). This is more evident on blast T cells growing in continuous cultures (9-11) and on the blast cells formed in mixed lymphocyte culture (MLC)¹ reactions (12, 13). The source of the Ia antigens on the blast cells appears to come from the T cells but this is not entirely settled because Ia antigens from stimulatory cells have been shown to adhere to responding T cells (14).

In the present study, T-cell blasts were produced by a wide variety of mitogens as well as a number of antigens. In all instances, Ia-positive T-cell blasts were produced which were shown to be characteristic of the responding T cell. A preliminary report of these findings has been presented (15).

Materials and Methods

Lymphocyte Preparation. Peripheral blood mononuclear cells were obtained by Ficoll-Hypaque density gradient centrifugation. Spontaneous rosettes with neuraminidase-treated sheep erythrocytes (E rosettes) were used for T-cell isolation. After repeated density gradient separation on Ficoll (Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, N. J.)-Hypaque (Winthrop Laboratories, New York), T cells were freed from erythrocytes hypotonic lysis. This fraction contained >95% E rosette-forming cells on rosetting with sheep erythrocytes. T

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Abbreviations used in this paper: BSA, bovine serum albumin; Con A, concanavalin A; E rosette, sheep erythrocyte rosette; FCS, fetal calf serum; MLC, mixed lymphocyte culture; PHA, phytohemagglutinin; PPD, purified protein derivative; PWM, pokeweed mitogen.

lymphocytes were also prepared by depletion of complement receptor⁺ cells (16). The initiation and maintenance of lymphoblastoid B cell lines have been described (17).

Culture Systems. Mitogen stimulation was performed on Linbro tissue culture plates (Flow Laboratories, Inc., Rockville, Md.) at 37°C in 5% CO₂ and 95% air. Lymphocytes at 1×10^6 /ml were cultured in 2-ml volumes of RPMI-1640 with 20% FCS, 2 mM glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin, 2.5 µg/ml amphotericin B, and 25 mM HEPES buffer (all from Grand Island Biological Co., Grand Island, N. Y.). Pokeweed mitogen (PWM) and phytohemagglutinin (PHA) (both from Grand Island Biological Co.) were used at a final concentration of 1:100; Concanavalin A (Con A) (Pharmacia Fine Chemicals, Div. of Pharmacia Inc.) at 40 µg/ml. Similar cultures were made in 0.2-ml volumes and DNA synthesis measured after [³H]thymidine (Schwarz/Mann Div., Becton, Dickinson, & Co., Orangeburg, N. Y.) incorporation for 16 h.

Stimulation with tetanus toxoid (State Laboratory Institute, Jamaica Plains, Mass.) and purified protein derivative (PPD) (Parke-Davis, Div. of Warner-Lambert Co., Morris Plains, N. J.) was performed with peripheral blood mononuclear populations or T cells supplemented with irradiated (3,000 R) adherent cells in RPMI-1640 with 10% pooled human AB serum, glutamine, penicillin, streptomycin, amphotericin B, and HEPES buffer as detailed above. The final concentration of tetanus toxoid was 40 µg/ml and that of PPD was 15 µg/ml.

Allogeneic one way MLC were made in RPMI-1640 with 10% pooled AB serum. Isolated T cells were used as responders. Irradiated peripheral blood mononuclear cells were used as stimulators in primary MLC reactions and irradiated E rosette-depleted cells used as stimulators in secondary MLC. Cell concentration at initiation of cultures equaled 1.5×10^6 /ml, with one-half responder and one-half stimulator populations.

Supernates of mononuclear cell cultures with PHA were obtained and T-cell cultures maintained in conditioned medium as described by Gillis et al. (10).

Antiserums. The use of heteroanti-Ia for immunofluorescence and depletion of Ia⁺ cells has been described in detail (9). Antiserums against heavy and light chains of immunoglobulins were prepared and used as before (17). Alloantiserums were obtained from multiparous women and renal transplant recipients and used after absorption with T-cell lines and platelets. Indirect immunofluorescence and cytotoxicity assay for HLA-DR specificities were performed as described previously (1, 18). Double-marker studies were made by alloantiserum staining (rhodamine) followed by heteroanti-Ia staining (fluorescein).

Results

Ia Expression on Mitogen-Stimulated T Lymphocytes. When peripheral blood T cells were isolated by the E rosette method and then cultured with mitogens, they underwent blast transformation and Ia antigens gradually became detectable on their surfaces. The onset and peak of blastogenesis as shown by morphology on Wright's staining of cytocentrifuge preparations or reflected by [³H]thymidine uptake occurred earlier in the course of culture than those of Ia staining (by 1–5 d, depending on the mitogen used). Expression of Ia antigens usually became evident only after 4–6 d of culture. A typical example is illustrated by the PWM culture shown in Fig. 1. This delay is even more obvious in the Con A culture shown in Fig. 2. The PHA-cultured T cells in Fig. 3 also showed a delay in maximal Ia expression.

Blasts were isolated on a bovine serum albumin (BSA) density gradient. It was found that a variable proportion of them were Ia⁺. In an experiment using T cells cultured for 6 d with Con A, only 42% of blasts thus isolated were stained positive with heteroanti-Ia antiserum. Isolation of T-cell blasts from PWM cultures showed a high percentage of Ia-positivity (>90% in some instances). However, with all the mitogens used, practically all nonblasts were Ia⁻.

The staining of mitogen-stimulated T cells from three individuals for Ia antigens is shown in Table I. In general, PWM stimulation yielded the highest percentage of Ia⁺

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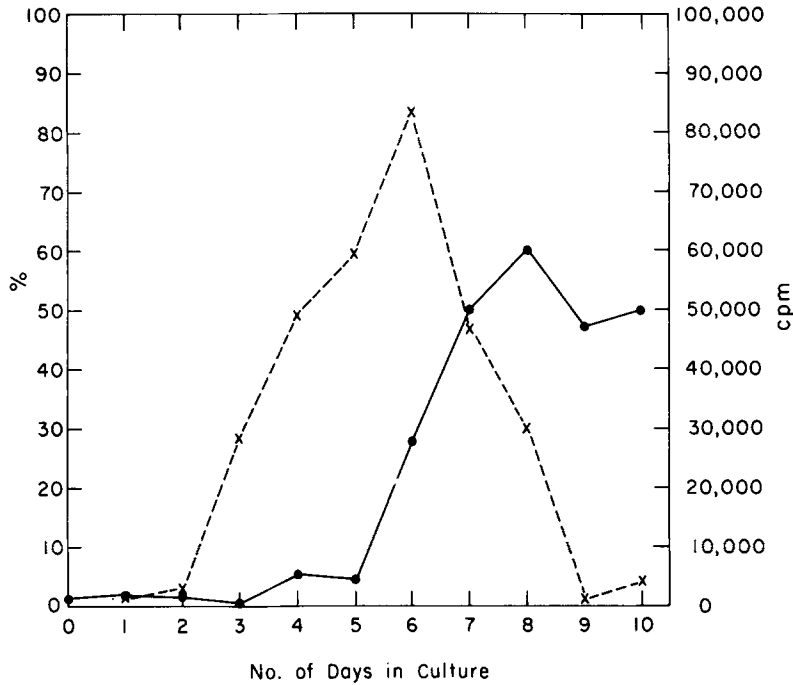


FIG. 1. Effect of PWM stimulation on the proliferative response and percentage Ia expression of T cells by fluorescent analysis with heteroanti-Ia serum. Isolated peripheral blood T cells were cultured at 1×10^6 /ml in RPMI-1640 with 20% FCS and PWM (1:100). [³H]thymidine uptake was determined 16 h after pulsing each culture with 2 μ Ci of labeled thymidine. x---x, [³H]thymidine uptake; ●—●, Ia⁺ cells.

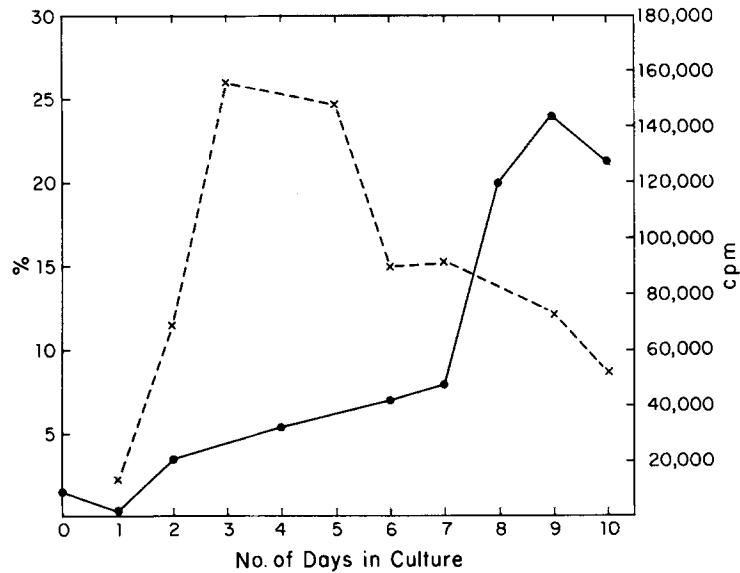


FIG. 2. Effect of Con A stimulation on the proliferative response and percentage Ia expression of T cells by fluorescent analysis with heteroanti-Ia serum. Conditions the same as in Fig. 1 except for the addition of Con A (40 μ g/ml) as mitogen. x---x, [³H]thymidine uptake; ●—●, Ia⁺ cells.

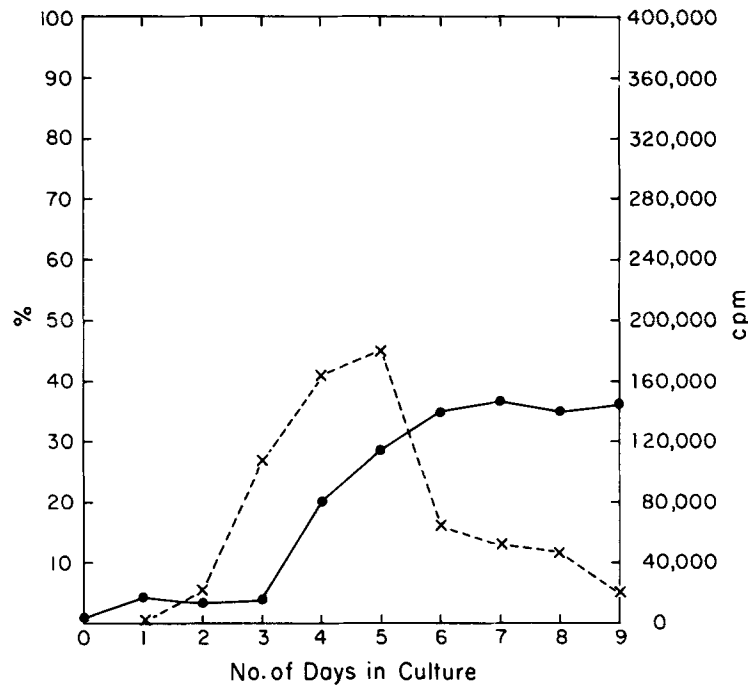


FIG. 3. Effect of PHA stimulation on the proliferative response and percentage Ia expression of T cells by fluorescent analysis with heteroanti-Ia serum. Conditions the same as in Fig. 1 except for the addition of PHA (1:100) as mitogen. ×---×, [³H]thymidine uptake; ●—●, Ia⁺ cells.

TABLE I
Expression of Ia on Mitogen-Stimulated T Cells from Three Donors

Donor	Ia ⁺ cells in culture with		
	PHA	Con A	PWM
		%	
A	15	31	60
B	—	16	43
C	29	30	33

Isolated T cells were cultured in RPMI-1640 with 20% FCS supplemented with antibiotics and glutamine. Cell concentration = 1×10^6 /ml. Mitogen concentrations: PHA = 1:100, PWM = 1:100 and Con A = 40 μ g/ml. After 7 d of culture in 5% CO₂ at 37°C, cells were harvested and stained with rhodamine-conjugated rabbit anti-human Ia.

cells (mean \pm SD = 47.1 \pm 12.7%). These cells were shown to be E rosette⁺ and rarely positive for surface or intracellular immunoglobulin (Table II). The percentage of Ia⁺ cells with PHA and Con A cultures was more variable and usually lower (mean \pm SD = 29.6 \pm 14.5% for PHA and 26.9 \pm 15.3% for Con A). The intensity of staining varied from weakly to strongly positive in PWM-stimulated cells but was often much weaker in PHA and Con A blasts. However, when stimulated T cells were put into continuous growth with conditioned medium from PHA culture supernates (8), all the cultured cells became blasts and >90% were stained positive for Ia 2 wk after initial stimulation. Blasts generated by Con A and PHA also became brightly

TABLE II
Surface Markers of PWM-Stimulated T Cells

Donor	Cells positive for			
	E rosette	Ia	sIg*	cIg‡
			%	
D	96	27	<0.01	<0.01
E	97	44	<0.01	1.1
F	96	33	0.8	0.8

Isolated T cells were cultured for 8 d with PWM. Culture condition same as in Table 1.

* sIg, surface immunoglobulin.

‡ cIg, cytoplasmic immunoglobulin.

stained for Ia antigens after further culture in such medium. In all instances, control cultures without mitogens consistently yielded very low percentages of staining. Stimulated T cells initially isolated by depletion of complement receptor⁺ cells showed Ia staining comparable to those isolated by the E rosette method. Absorption of the heteroanti-Ia serum with B cells abolished the staining on T-cell blasts. Conversely, T-cell blasts removed the Ia staining of B cells and B-cell lines.

Effect of Anti-Ia and Complement on Subsequent Ia Expression on T Lymphocytes. In the majority of the isolated T-cell preparations, 2–4% were found to be Ia⁺ (9). The role of this small population of Ia⁺ T cells in the generation of Ia⁺ T blasts upon activation was assessed. Thus, isolated T lymphocytes were treated with anti-Ia and complement. After washing, they were put into culture with PWM. The efficacy of depletion of Ia⁺ cells was confirmed by the subsequent lack of allogeneic helper activity from this treated population (9). In one experiment, 18% of the cells so treated expressed Ia after 7 d of culture, whereas 41% of cells showed positive staining in a PWM culture of the untreated cells, i.e., a decrease by 23% with the treated sample. In a control culture, cells treated with normal rabbit serum and complement developed similar staining as the untreated group (38%). Thymidine uptake among the three cultures was not significantly different. In two additional experiments decreases by 19.2% (32% with control vs. 12.8% with treated sample) and 16% (40% vs. 24%) were found after anti-Ia treatment.

Ia Expression on T Lymphocytes Stimulated with Soluble Antigens. Peripheral blood mononuclear cells from tetanus toxoid responders were cultured with 40 µg/ml tetanus toxoid. In a representative experiment (Fig. 4), blastogenesis as represented by [³H]thymidine uptake became maximal on the 5th d of culture. Positive staining for Ia was noted throughout the course because B cells and monocytes were initially present. On the 6th d, Ia staining rose above control level (24%) and reached 54% on day 7. Double-marker study at this point showed 44% of E rosette⁺ cells were Ia⁺. Isolated T cells cultured with tetanus toxoid in the presence of different doses of irradiated adherent cells gave findings similar to those with unseparated mononuclear cells. With mononuclear cells from a nonresponder who gave <10,000 cpm in response to tetanus toxoid stimulation, rare Ia⁺ E rosette⁺ cells were found at the end of a 7-d culture.

The staining of mononuclear cells from a PPD responder for Ia antigen after a 7-day culture with 15 µg/ml of PPD was found to be 70%. 50% of the E rosette⁺ cells

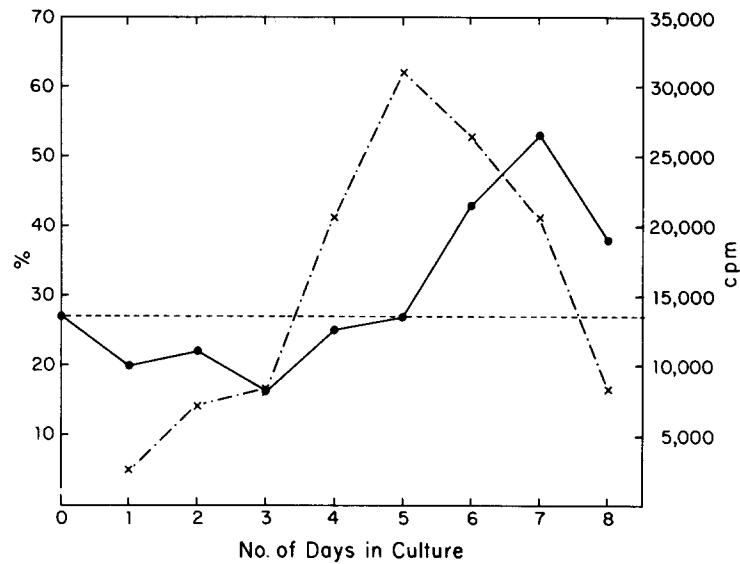


FIG. 4. Effect of tetanus toxoid stimulation on the proliferative response and percentage Ia expression of peripheral blood mononuclear cells from a tetanus toxoid responder by fluorescent analysis with heteroanti-Ia serum. Peripheral blood mononuclear cells were cultured at 1×10^6 /ml in RPMI-1640 with 10% AB serum and tetanus toxoid ($40 \mu\text{g}/\text{ml}$). The broken line (---) indicates level of Ia⁺ cells on Day 0. [³H]thymidine uptake was determined as in Fig. 1. ×—×, [³H]thymidine uptake; ●—●, Ia⁺ cells.

were Ia⁺. Isolated cells from the same individual cultured in the presence of irradiated autologous adherent cells were 41% Ia⁺ on the 7th d and 97% of cells in this culture formed E rosettes.

Ia Expression on T Lymphocytes Stimulated by Allogeneic Cells. MLC reactions were made one way by using T cells as responders and irradiated phagocyte-depleted mononuclear cells as stimulators. Ia staining gradually rose above background level and reached a peak on about the 7th–8th d. In a typical experiment (Fig. 5), the maximum staining reached on the 7th d was 61% when the proportion of E rosette⁺ cells was 92%. When the cells were examined on cytocentrifuge preparations after Wright's staining, it was observed that although blastogenesis started before the rise of Ia above base-line level, it peaked at about the same time as maximal Ia expression. The proportion of Ia⁺ cells declined with reversion of blasts to small lymphocytes. On secondary MLC stimulation, the Ia staining of responder T cells was found to peak again as blastogenesis recurred (Fig. 5). Subsequent decline after reversion was also noted.

Detection of Ia on Stimulated T Cells Using Alloantisera. When stimulated T cells were stained for Ia with alloantisera, the pattern of staining in general corresponded to that of cytotoxicity assay on freshly separated B cells. This was further confirmed by the staining on autologous B-cell lines which have proven of special utility in fluorescent analysis for specific alloantigens. In a representative experiment, T cells were obtained from a donor whose B cells were typed to be positive for HLA-DRw 2 and -DRw 3 by cytotoxicity and cultured with PWM for 6 d. The autologous B-cell line was stained by alloantisera of -DRw 2 and -DRw 3 specificity. The cultured

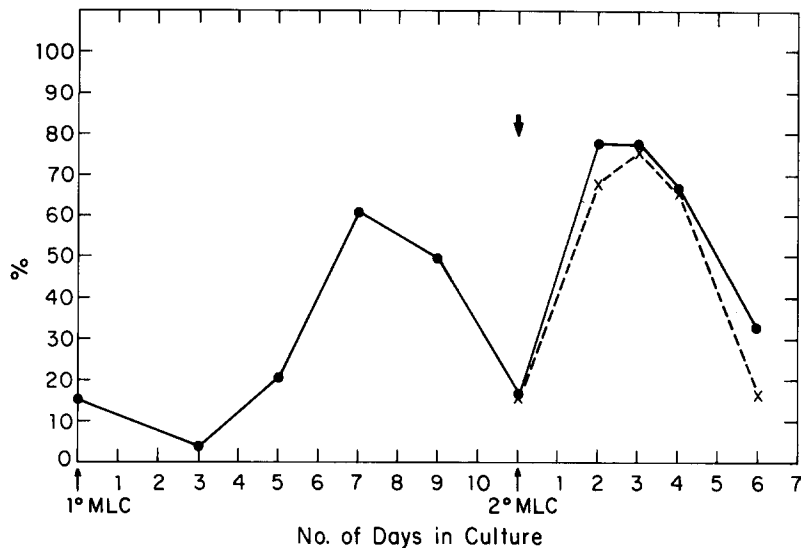


FIG. 5. Ia expression during a primary and secondary MLC reaction using heteroantiserum and alloantiserum specific for the responder cells. During the secondary response, a similar high percentage of the responding T cells were positive by both antisera. Isolated responder T cells (1.5×10^6 /ml) were mixed with irradiated stimulators (1.5×10^6 /ml) in RPMI-1640 with 10% AB serum at a ratio of 1:1. Stimulators were peripheral blood mononuclear cells in primary MLC and isolated non-T fraction in secondary MLC. Bold arrow indicates onset of secondary MLC. Separate cultures of the irradiated stimulator during the course of secondary MLC showed <15% viability from Day 2 on. ●—●, staining by heteroanti-Ia serum; x---x, staining by alloantiserum specific for responder HLA-DR.

T cells revealed appropriate specificities, i.e., -DRw 2 and -DRw 3. As shown in Table III, the cells bearing these allo-determinants also were stained positive by heteroantiserum in double-labeling experiment.

A special search for alloantigens on Ia-bearing T blasts not found on B cells from the same individual was made using a large number of alloantisera from transplant patients and pregnant women. Thus far, such antigens have not been found. Alloantisera of unrelated specificities usually did not stain the stimulated T cells. Occasional discrepancies such as that given by serum 191 (Table III) were noted. This serum detected DRw 4 × 7 alloantigen and did not react with fresh T cells or B cells of the donor mentioned above. Nevertheless, it stained a small proportion of the stimulated T cells among both positively and negatively stained fractions by heteroantiserum. However, the staining with such sera was much weaker than that given by sera of appropriate specificities.

Mitogen and antigen stimulation to produce blasts did not introduce a foreign Ia source and therefore, these experiments were the most revealing especially in view of the alloantigens expressed. In continuous T-cell cultures and MLC experiments, it was necessary to document the source of surface Ia on the blasts. For example, T cells from a donor of HLA-DRw 2 phenotype were cultured with PHA-conditioned medium from a second donor of HLA-DRw 3. On the 13th d of culture when the cells were typed with alloantisera, they were found to be selectively stained for DRw 2 but not DRw 3. Similar findings were obtained in a number of additional experiments. In MLC reactions, both heteroantiserum and alloantiserum specific for responder -DR phenotype stained similar proportions of cells during the course of

TABLE III
Expression of -DR Alloantigens on B Cells and PWM-Stimulated T Cells
 (Donor B Cells -DRw 2 and 3)

Alloantiserum (and specificity)	Autologous B-cell line	Positive cells with alloantiserum staining	
		6th d cultured T cells whose staining by heteroanti-Ia was	
		Positive	Negative
		%	
1146 (DRw 2)	95	98	<1
932 (DRw 3)	97	99	2
1995 (DRw 5)	<1	<1	<1
191 (DRw 4 × 7)	—	9.4	25

T cells were cultured with PWM at 1×10^6 /ml for 6 d. Cells were stained first with alloantiserum by indirect method using rhodamine-conjugated goat anti-human Fc γ . After washing, they were counterstained with fluorescein-conjugated rabbit anti-human Ia.

culture. This was especially striking in secondary MLC reactions (Fig. 5). In this experiment, 75% of the cells stained for Ia with both the hetero- and specific responder alloantisera. However, transitory positive staining for stimulator -DR phenotype has also been encountered. Further details of these studies with specific alloantisera will be reported separately.

Discussion

The present studies demonstrate that Ia antigens are found on T cells stimulated with both mitogens and antigens. Among the three mitogens used, PWM seems to be most effective in giving a high proportion of Ia⁺ T cells in culture. These cells are shown to lack other B-cell markers. PHA and Con A usually give fewer and weaker positive cells despite better thymidine uptake. Also, the kinetics of Ia expression differs from that of blastogenesis. There is usually a lag in Ia appearance. Almost all Ia⁺ cells appear to be blasts. In addition, Ia⁻ blasts can be observed especially at the peak of blastogenesis. In these experiments isolated T cells were used; in the soluble antigen stimulation systems, peripheral blood mononuclear populations were used because T cells alone respond poorly without some adherent cells. Evidence was obtained that with 7-d cultures of tetanus toxoid or PPD, 40–50% of the cultured T cells from a responder were Ia⁺. However, similarly cultured T cells from a nonresponder remained Ia⁻. Thus, all the mitogen and antigen systems employed yielded blast cells that expressed Ia antigens as detected both by hetero- and allo-antisera. The specific DR alloantisera demonstrated that the same antigens were expressed on the stimulated T cells as on the B cells of a given individual. Extensive studies of this type indicated that it was practical to type different subjects on the basis of the T-cell blast reactivity. These results and certain spurious reactions that were occasionally found will be reported in detail separately.

Careful removal of essentially all B cells and monocytes resulting in the use of highly purified T cells especially in the mitogen experiments and the above results with the Ia alloantisera provide strong evidence for the production of the Ia antigens by the stimulated T cells. Additional support for this conclusion was obtained from internal labeling with [³⁵S]methionine. Synthesis of the Ia bimolecular complex

by mitogen-stimulated T cells was documented (C. Y. Wang, unpublished data). The continuous T-cell cultures and the MLC systems represent a somewhat different problem. In view of the results of Nagy et al. (14) which showed binding of stimulator alloantigens to MLC-activated murine T blasts, experiments were done to examine if stimulated T cells took up Ia antigens from culture supernates. Uptake of Ia was not demonstrable using PHA-conditioned medium. However, transitory uptake of stimulator cell -DR antigens was observed in MLC reactions.

The present studies as well as those reported previously (9) documented that a small population of peripheral blood T cells in man bore Ia antigens. Whether these Ia⁺ T cells are a result of in vivo activation of T cells remains undetermined. A recent study reporting that a much higher number of Ia⁺ T cells were found in circulation during the acute phase of infectious mononucleosis would appear to support this possibility (19). However, evidence was obtained that at least the majority of the normal Ia⁺ cells were not blasts. Experiments using anti-Ia and complement to deplete freshly isolated Ia⁺ T cells resulted in a consistent decrease in the subsequent proportion of Ia⁺-stimulated T cells. This suggests that Ia-bearing T cells from peripheral blood were precursors of some of the Ia⁺ blasts or their presence is necessary for optimal cell growth and subsequent Ia expression. In contrast to B cells, which on differentiation lose Ia, activated T cells acquire these antigens. However, the presence of Ia⁻ T-cell blasts and the fact that the intensity of Ia staining varies among the stimulated cells indicate that the blast population is heterogeneous. Furthermore, blasts from T-cell acute lymphoblastic leukemia and lymphoblastoid T-cell lines are in general Ia⁻. Thus, the exact functions of the newly expressed Ia antigens on these T-cell blasts remain unknown.

Summary

Human T-cell blasts were generated by stimulation with mitogens and antigens. A proportion of these blasts expressed Ia antigens detectable by immunofluorescence with both allo- and hetero-antisera. The maximal expression of Ia antigens was delayed and usually occurred after the peak of blastogenesis. Among the three mitogens used, pokeweed mitogen (PWM) was most effective in giving a high percentage and intense Ia staining of T-cell blasts. Phytohemagglutinin and concanavalin A blasts gave weaker and lower percentages of Ia staining. Activation by alloantigens and soluble antigens such as tetanus toxoid and purified protein derivative resulted in Ia expression on T cells comparable to PWM stimulation. Depletion of Ia⁺ cells from freshly isolated T cells with anti-Ia and complement decreased subsequent Ia expression, suggesting that a proportion of Ia⁺ blasts were derived from Ia-bearing peripheral blood T cells.

When the specificities of the Ia antigens on T-cell blasts were examined with alloantisera, it was evident that the T blasts expressed similar HLA-DR determinants to those on B cells from the same donor; occasional minor differences between stimulated T cells and autologous B-cell lines or fresh B cells were encountered.

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