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Research Article

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Recognition of Hapten-Modified Cells In Vitro by Human T Lymphocytes

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ABSTRACT Clearer definition of the recognitive structures of human T lymphocytes for antigens will be required to elucidate the molecular basis of diseases and immunological responses induced or regulated by normal or abnormal T-cell function. For this purpose we have investigated the cellular requirements for immune responses in vitro to trinitrophenyl-conjugated peripheral blood mononuclear cells. The responding cell was characterized as a T cell on the basis of rosetting with sheep erythrocytes. T-cell recognition of hapten in proliferative responses depended upon presentation of antigen in an appropriate stimulator-cell context. Neither autologous hapten-modified erythrocytes nor T cells restimulated responses of in vitro-primed lymphocytes. Moreover, hapten-conjugated non-T cells were more effective than modified unfractionated cells in restimulating proliferative responses. Both macrophages and non-T lymphocytes effectively restimulated hapten-conjugate responses.

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INTRODUCTION

Specific abnormalities of human T-cell activation or control of immune responses seem critically important in the pathogenesis of numerous diseases. The increasing recognition of the association of HLA antigens with a predisposition to certain diseases (1, 2) has focused urgent attention on elucidation of the function of major histocompatibility complex (MHC)[1] genes in the pathogenesis of such disorders. Furthermore, the development and course of disease processes induced by immune responses to modifications in autologous cell surfaces may be controlled by genes within the MHC. An understanding, however, will first require a definition of the role of histocompatibility determinants in T-cell recognition. Examination of human in vitro proliferative and cytotoxic responses to hapten-modified autologous cells may allow a functional characterization of the autologous determinants involved in both normal and abnormal immune responses.

In the mouse, recognition of viruses (3), minor histocompatibility antigens (4) and haptenic determinants (5–7) by T cells is dependent upon the expression of autologous determinants controlled by the murine MHC, *H-2*. Thus, in vitro T-cell function is restricted in cytotoxic responses by H-2K and H-2D antigens, and in proliferative responses by H-2I-region-encoded Ia antigens. In vivo, immune T cells can only transfer resistance to virus-infected hosts if *H-2K* or *H-2D* homology requirements are met (8). On the other hand, resistance to mycobacterial infections are only conferred by T cells which share H-2I-region determinants (thought to be analogous to the HLA-D region) with the host (9). In addition, the expression of Ia determinants on guinea pig macrophage subpopula-

[1] *Abbreviations used in this paper*: Δcpm, net counts per minute; MHC, major histocompatibility complex; PBMC, peripheral blood mononuclear cell(s); TNP, trinitrophenyl.

tions has been shown to be critical in the presentation of both protein antigens and hapten-modified cell surface determinants to responder T cells (10–12).

Recent studies in humans have demonstrated that cell-mediated cytotoxic responses to the Y male antigen (13), influenza-infected cells (14, 15), and hapten-modified cells (16, 17) are constrained by HLA-A- or -B-locus antigens. In contrast, our previous data (18, 19), recently confirmed by Thorsby and Nousiainen (20) indicated that secondary proliferative responses of human peripheral blood lymphocytes to hapten-modified cells were associated with the *HLA-D* region. Nevertheless, a substantial portion of the proliferative response to hapten-modified human cells was not dependent upon the expression of typed histocompatibility antigens. Thus, hapten-modified allogeneic stimulator cells that did not share major histocompatibility antigens with sensitized responders often induced substantial proliferative responses. To better understand the role of autologous determinants in the recognition of antigen by human lymphoid cells, we have characterized the cellular components involved in responses to hapten-modified cells. Human lymphocytes were primed in vitro to autologous trinitrophenyl (TNP) -conjugated stimulator cells and restimulated with various hapten-modified cell populations. The data strongly suggest that human T-cell recognitive units for stimulation of secondary proliferative responses, in contrast with those involved in restimulation of primed cytotoxic responses, are dependent upon the presentation of hapten in the context of macrophage or non-T-lymphocyte surface antigens.

METHODS

Cell culture technique. Heparinized venous blood was obtained from healthy human subjects and peripheral blood mononuclear cells (PBMC) were isolated and conjugated to TNP with trinitrobenzene sulfonic acid (ICN Nutritional Biochemicals, Cleveland, Ohio) as previously described (18). Cells were cultured in RPMI 1640 (Grand Island Biological Co., Grand Island, N. Y.) supplemented with 20% AB-positive, heat-inactivated, pooled human plasma.

For primary stimulation, cells were cultured in round-bottom 17 × 100-mm tubes (2001, Falcon Labware, Oxnard, Calif.). Responder and stimulator cells were each cultured at a final concentration of 7.5 × 10^5 cells/ml in 2-ml cultures for 20–30 d as previously described (18). Stimulator cells were inactivated with 500 rads of ^{60}Co (500 rads/min).

For secondary stimulation, primed cells were harvested, washed, and cocultured in round-bottom microculture wells (IS-MRC-96; Linbro Chemical Co., Hamden, Conn.) for assessment of proliferative responses, or in 17 × 100-mm round-bottom tubes for induction of cytotoxic effector cells. Responder cells (1.5 × 10^5/microculture well or 1.5 × 10^6/tube) were cultured with an equal number of 2,000 rad-inactivated fresh TNP-conjugated or unconjugated stimulators.

Glutaraldehyde-fixed stimulator cells were prepared according to the method of Forman (21). TNP-conjugated or unconjugated cells suspended in phosphate-buffered saline (pH 7.4) were added to an equal volume of glutaraldehyde (Polysciences, Inc., Warrington, Pa.) in phosphate-buffered saline. The cells were treated for 10 s before addition of a 50-fold excess of Hanks' balanced salt solution (Microbiological Associates, Walkersville, Md.).

Proliferative responses to TNP-conjugated cells. Proliferative responses were assessed on days 3 or 4 after secondary stimulation by addition of 1.0 μCi of tritiated thymidine (2.0 Ci/mM sp act, New England Nuclear Corp., Boston, Mass.) to cultures for the final 20 h of the incubation period.

Data from separate experiments are expressed as mean counts per minute of three to four replicate cultures with the SEM. Net counts per minute (Δ cpm) were calculated by subtracting the counts per minute of responses to unconjugated stimulators from the counts per minute of cultures with TNP-conjugated stimulator cells. Errors for Δ cpm calculations were determined by the formula for propagation of error. Percent control responses were calculated by determining the mean response to stimulators and employing the following formula: ([(cpm experimental TNP-conjugated) − (cpm experimental unconjugated)]/[(cpm unfractionated TNP-conjugated) − (cpm unfractionated unconjugated)]) × 100.

Cytotoxic responses to TNP-conjugated cells. Cytotoxic responses were assessed using a ^{51}Cr-release assay. Primed effector cells were harvested from tubes 4–5 d after secondary stimulation, washed, and adjusted to appropriate concentrations in RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum (Grand Island Biological Co.). The target cells for the cell-mediated lympholysis assay were PBMC that had been cultured for 4–5 d. These cells were harvested on the day of assay, labeled with ^{51}Cr by incubating 30 × 10^6 cells for 60 min with 300 μCi of $Na_2{}^{51}CrO_4$ (New England Nuclear Corp.), and either TNP-conjugated or left unmodified. All target cell preparations were washed three times before use in the assay. 20,000 target cells were then incubated in 0.2 ml of RPMI 1640 supplemented with 10% fetal calf serum in triplicate cultures with varying numbers of viable effector cells in 12 × 75-mm plastic tubes (Falcon Labware). After incubation for 6 h at 37°C in a humidified, 5% CO_2 atmosphere, ^{51}Cr-release was measured as previously described (22). Cytolytic activity is expressed as: % specific release = ([experimental release − spontaneous release]/[freeze-thaw − spontaneous release]) × 100. Spontaneous release values were consistently <25% of total counts per minute.

Identification of lymphoid cell populations. The percentage of human T cells in various fractions of PBMC was assessed by a standard sheep erythrocyte E-rosetting technique (23). C3 receptor-bearing cells were detected by rosette formation with sheep erythrocytes coated with 19S anti-sheep antibodies (Cordis Labs. Inc., Miami, Fla.) and mouse complement (23). Numbers of monocytes and macrophages were estimated with nonspecific esterase staining (24). Surface immunoglobulin-positive cells were assayed with a fluorescein-conjugated goat anti-human polyvalent antisera (Behring Diagnostics, Somerville, N. J.) according to method of Pernis et al. (25).

Cell fractionation procedures. Unfractionated PBMC contained ≅65% E rosette-positive cells (T cells), 35% C3 receptor-bearing cells (macrophages and B cells) and 25% esterase-positive cells (macrophage-monocytes). To obtain T-cell and non-T-cell lymphoid populations a rapid sheep erythrocyte-rosette procedure was used (26). Briefly, PBMC were rosetted with neuraminidase (20 U/ml Receptor Destroying Enzyme-Vibrio comma extract, Microbiological Associates) treated sheep erythrocytes for 30 min at 4°C and E rosette-positive and non-E-rosette fractions isolated by Ficoll-Isopaque (Pharmacia Fine Chemicals Inc., Piscataway, N. J.) gradient centrifugation. Sheep erythrocytes were lysed

with Tris/NH_4Cl, and cells were washed twice with media. Unfractionated controls were treated in the same manner. The non-E-rosette population contained <8% E rosette-positive cells, >40% esterase-positive cells, and >60% C3 receptor-bearing cells. To obtain a T cell-enriched population the E-rosette fraction was rosetted a second time. The double E rosette-purified cells contained ≅85% E rosette-positive cells, <2% esterase-positive cells, <5% C3 receptor-bearing cells, and <2% surface immunoglobulin-positive cells. All populations were >95% viable as assessed by trypan blue.

Macrophage-monocyte populations were isolated by a plastic dish adherence procedure. 50 million PBMC were incubated in 100 × 20-mm plastic Petri dishes (Falcon Labware) for 45 min at 37°C in a 5% CO_2 incubator in 10 ml of RPMI 1640 with 10% autologous serum that had not been heat inactivated. Nonadherent cells were removed from Petri dishes by vigorously washing four times at 37°C with 7 ml of RPMI 1640 with 20% fetal calf serum. To recover adherent cells, Petri dishes were then incubated at 37°C for 30 min in the presence of 3 mM of EDTA and then washed twice with Hanks' balanced salt solution at 4°C. The recovered cells were >95% esterase positive and >95% viable. To control for possible adverse effects of EDTA treatment, both adherent and nonadherent cell populations were treated with EDTA in identical fashion in experiments in which adherent cells were isolated.

To obtain non-T lymphocytes, cells that did not adhere to plastic Petri dishes were first fractionated into E rosette-positive and -negative populations. E rosette-negative cells were then depleted of monocytes by passage thru Sephadex G-10 (Pharmacia Fine Chemicals Inc.) columns using a modification of the method of Ly and Mishell (27, 28). The resultant population contained <10% E rosette-positive cells, <5% esterase-positive cells, and >40% surface immunoglobulin-positive cells.

HLA typing. Typing for HLA-A- and -B-locus antigens was performed in the Methodist Hospital Tissue Typing Laboratory with standard serologic methods (29). HLA-D-region specificities were similarly detected with B-cell typing procedures (DRw) (30).

RESULTS

Characterization of human TNP-conjugate responder cells. The ability of human T cells to form rosettes with sheep erythrocytes was used to determine the responder cell in proliferative responses to hapten-modified cells. Unfractionated PBMC were sensitized to autologous hapten-modified cells and 3 wk later separated into E-rosette and non-E-rosette cell populations. As shown in Table I, the unfractionated, E rosette-positive and E rosette-negative primed responder cells were then restimulated with autologous PBMC which were either TNP-conjugated or unconjugated. The E rosette-positive cells were clearly better responders than the unfractionated cells. Conversely, the responses of the non-E-rosette cells were substantially reduced. The small response noted by the latter population may be explained by the residual contamination, ≅8%, of E rosette-positive cells, although modest intrinsic responsiveness of the non-E-rosette population was not ruled out. These experiments thus demonstrate that the cells which proliferated in response to hapten-conjugated PBMC were largely or exclusively T lymphocytes.

TABLE I
Characterization of the Responder Cell Population in Secondary Hapten-Conjugate Proliferative Responses

Responder cells*	Unconjugated‡	TNP-conjugated	Δcpm§	Percent control‖
Experiment 1				
Unfractionated	1588±147¶	17,799±721	16,211±735	100
E-rosette	1542±206	27,695±1218	26,153±1235	161
Non-E-rosette	3460±150	7,739±385	4,279±413	26
Experiment 2				
Unfractionated	802±41	9,214±50	8,412±64	100
E-rosette	2522±130	15,633±872	13,111±882	156
Non-E-rosette	894±124	3,304±223	2,410±255	29

* Primed responder cells were generated by co-culture with autologous TNP-conjugated stimulators for 3–4 wk. These sensitized cells were either left unfractionated or separated into E rosette-positive or non-E-rosette responder populations. Responder cells were cultured at 1.5 × 10^5 cells/microculture well.

‡ TNP-conjugated or unconjugated stimulators were fresh PBMC inactivated with 2,000 rads and cultured at 1.5 × 10^5 cells/microculture well together with responder cells. Cultures were harvested 70 h after secondary stimulation.

§ Δcpm were calculated by subtracting counts per minute of responses to unconjugated stimulators from counts per minute of cultures with TNP-conjugated stimulators.

‖ Calculated by formula given in Methods.

¶ Data expressed as mean counts per minute of three to four replicate cultures with the SEM.

Characterization of human TNP-conjugate stimulator cells. Previous data from our laboratory suggested that human lymphocytes, at least in part, recognize haptens in the context of autologous MHC determinants. Lymphocytes primed to autologous TNP-modified cells are preferentially restimulated by TNP-modified cells that share HLA-D-region determinants. However, a substantial portion of the secondary TNP-conjugate proliferative response did not appear to be genetically restricted. To examine whether a component of secondary proliferative responses was not dependent upon autologous histocompatibility antigens on lymphoid cells, primed responder cells were restimulated with hapten-modified autologous erythrocytes. Erythrocytes express only low levels of HLA-A- and -B-locus determinants and do not express detectable D-region antigens (31). As shown in Table II, TNP-conjugated erythrocytes did not restimulate proliferative responses. In experiments not shown, erythrocyte stimulator concentrations up to six times that of mononuclear cells were still ineffective in inducing responses. These results are consistent with the concept that expression of HLA determinants is necessary for the induction of proliferative responses to haptens. Furthermore, as shown in Table II, viable stimulator cells are required. Thus, neither heat-killed cells nor glutaraldehyde-fixed cells restimulated TNP-primed responder cells. Stimulator cells treated with as little as 0.1% glutaraldehyde for 10 s were almost devoid of immunogenic activity.

Different subpopulations of lymphoid cells express varying amounts of histocompatibility antigens. T cells, B cells, and monocytes all express substantial quantities of HLA-A- and -B-locus antigens. However, only monocytes-macrophages, B cells, and "null" lymphocytes, in contrast with resting T cells, express readily detectable *HLA-D*-region-encoded Ia-like antigens on their cell surface (32, 33). It was, therefore, of particular interest to investigate the ability of PBMC subpopulations to stimulate hapten-conjugate proliferative responses.

Lymphocytes were primed to autologous TNP-modified PBMC and restimulated with different populations of TNP-modified or unmodified stimulator cells as shown in Table III. Hapten-modified T cells (E-rosette fraction) were not effective stimulators. Moreover, hapten-modified non-T cells (non-E-rosette fraction), which contained both monocytes and non-T lymphocytes, restimulated proliferative responses more effectively than modified unfractionated cells.

The ineffectiveness of modified T cells to function as stimulators might be explained by a culture requirement for fresh macrophages. On the basis of nonspecific esterase staining, the E-rosette stimulator cell fraction contained <2% monocytes-macrophages whereas the non-E-rosette stimulator cell fraction possessed ≅50% monocytes-macrophages. Although the primed responder cells contained 25% esterase-positive cells, it is possible that accessory cell function in long-term cultures is defective. It was also possible that the inability of T cells to stimulate hapten-conjugate proliferative responses could be explained by a suppressive mechanism. To address these questions, cell-mixing experiments were performed (Table IV). In these experiments, stimulator populations were comprised of equal concentrations of E-rosette and non-E-rosette cells that were either TNP-conjugated or unconjugated. The addition of non-E-rosette cells did not enhance the stimulatory capacity of TNP-modified E-rosette cells. Furthermore, the addition of E rosette-

TABLE II

*Neither Hapten-Conjugated Erythrocytes nor Dead PBMC Stimulate Secondary Proliferative Responses**

Stimulators‡	Unconjugated	TNP-conjugated	Δcpm	Percent control
PBMC, 1.5 × 10^5/well	329±54	25,971±1,545	25,642±1,546	100
Erythrocytes, 1.5 × 10^5/well	98±8	475±90	397±90	2
Erythrocytes, 6.0 × 10^5/well	246±56	982±125	737±137	3
PBMC	1,304±124	9,780±74	8,476±144	100
PBMC, heat killed	197±23	525±13	328±26	4
PBMC	1,396±46	10,131±74	8,735±87	100
PBMC, 0.1% glutaraldehyde	1,067±358	2,443±391	1,376±530	16
PBMC, 0.2% glutaraldehyde	529±47	1,364±157	835±164	10
PBMC, 0.4% glutaraldehyde	906±207	1,295±308	389±371	4

* Basic experimental protocol and data analysis as in Table I. Unfractionated responder cells were primed by coculture with autologous TNP-conjugated stimulators.

‡ Stimulators were either autologous PBMC or autologous erythrocytes. PBMC were killed after TNP-modification by either a 30-min incubation at 56°C or glutaraldehyde fixation for 10 s.

TABLE III
*Stimulatory Capacity of Hapten-Modified E-Rosette and Non-E-Rosette Cells in Secondary Proliferative Responses**

Stimulators‡	Unconjugated	TNP-conjugated	Δcpm	Percent control
Experiment 1				
Unfractionated	802±41	9,214±508	8,412±510	100
E-rosette	620±60	793±173	173±183	2
Experiment 2				
Unfractionated	520±14	8,087±402	7,567±402	100
E-rosette	669±63	1,981±204	1,312±214	17
Non-E-rosette	2,842±372	13,846±876	11,004±952	145
Experiment 3				
Unfractionated	1,304±124	9,780±740	8,476±750	100
E-rosette	1,076±368	3,235±320	2,159±488	25
Non-E-rosette	3,877±163	15,349±525	11,472±550	135

* Basic experimental protocol and data analysis as in Table 1. Unfractionated responder cells were primed by coculture with autologous TNP-conjugated stimulators.

‡ Stimulators were either fresh PBMC (unfractionated), or fresh PBMC which were fractionated into E-rosette-positive and non-E-rosette populations.

positive cells to modified non-E-rosette cells did not inhibit responses to the latter population. The data therefore suggested that hapten-conjugated T cells are intrinsically ineffectual as stimulators of secondary proliferative responses.

Additional fractionation procedures were then used to further characterize the cell populations capable of effectively stimulating TNP-conjugate proliferative responses. Unfractionated peripheral blood was first separated into plastic-adherent and nonadherent populations. The adherent fraction was consistently >95% esterase positive, whereas the nonadherent fraction contained 10–15% esterase-positive cells. Nonadherent cells were further separated into E rosette-positive and non-E-rosette fractions, and the non-E-rosette population (35–40% esterase positive) was passed through Sephadex G-10 columns to remove monocytes-macrophages.

Purified plastic-adherent peripheral blood monocytes functioned effectively as hapten-conjugate stimulators (Table V). This adherent monocyte population was, however, less stimulatory than unfractionated cells. Interestingly, the non-E-rosette fraction, which had been depleted of the adherent population, was more effective than the adherent monocytes in stimulating hapten-conjugate proliferative responses. Removal of most of the esterase-positive cells from the non-E-rosette fraction by passage over Sephadex G-10 columns did not affect the stimulatory capacity. That is, a non-T-lymphocyte fraction, which contained <4%

TABLE IV
*Hapten-Conjugated T Cells do not Stimulate Secondary Proliferative Responses**

	Stimulators			cpm	Δcpm
	Unconjugated		TNP-conjugated		
Experiment 1	NER + ER			2,438±146	—
	ER	+	TNP-NER	13,814±1044	11,376±1054
	NER	+	TNP-ER	3,929±155	1,491±213
Experiment 2	NER + ER			4,119±236	—
	ER	+	TNP-NER	14,547±382	10,427±450
	NER	+	TNP-ER	6,945±456	2,825±513

* Basic experimental protocol and data analysis as in Table I.

‡ Stimulator population contained equal concentration of E-rosette (ER) and non-E-rosette (NER) cells that were either TNP-conjugated or unconjugated.

TABLE V

*Ability of Monocytes and Non-T Lymphocytes to Stimulate Secondary Hapten-Conjugate Proliferative Responses**

Stimulators‡	Unconjugated	TNP-conjugated	Δcpm	Percent control
Experiment 1				
Unfractionated cells	857±75	4,110±192	3,253±206	100
Monocytes	299±24	2,562±204	2,263±205	70
Non-E-rosette cells	1,097±131	6,974±663	5,877±676	181
E-rosette cells	284±15	987±52	703±54	22
Non-T lymphocytes	1,591±89	8,824±222	7,233±239	222
Experiment 2				
Unfractionated cells	1,167±198	10,268±696	9,101±724	100
Monocytes	841±156	6,530±325	5,689±361	63
Non-E-rosette cells	1,649±222	9,318±347	7,669±412	84
E-rosette cells	587±85	1,426±158	839±179	9
Non-T lymphocytes	1,489±265	8,942±496	7,453±562	82

* Basic experimental protocol and data analysis as in Table I. Unfractionated responder cells were primed by coculture with autologous TNP-conjugated stimulator.

‡ Stimulators were either fresh PBMC (unfractionated) or lymphoid populations fractionated from fresh PBMC. Monocytes were plastic adherent cells which contained >95% esterase-positive cells. Non-E-rosette and E-rosette fractions were obtained by separation of the plastic nonadherent cell population. Non-T lymphocytes were non-E-rosette cells passaged through Sephadex-G10 columns (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.) to remove monocytes and macrophages. The non-E-rosette population contained 35–40% esterase-positive cells, whereas the non-T-lymphocyte population contained <4% esterase-positive cells.

esterase-positive cells, was as stimulatory as the non-E-rosette fraction, which contained over 40% esterase-positive cells, and was more stimulatory than the adherent population which contained over 95% esterase-positive cells. It was still possible, however, that the residual macrophage contamination was responsible for the ability of this fraction to stimulate. A titration analysis of the stimulator cell populations was therefore performed (Fig. 1). A ninefold dilution of the non-T-lymphocyte stimulator cells, containing <400 monocytes, stimulated responses as great as

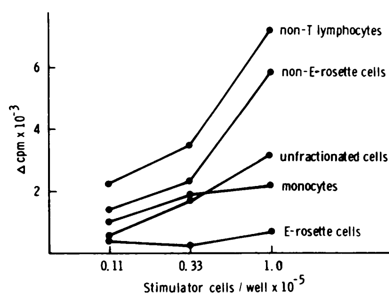

FIGURE 1 Titration analysis of proliferative responses to different hapten-conjugated subpopulations of PBMC. Primed responder lymphocytes were restimulated with various concentrations of stimulator cells obtained as described in Table V.

>95,000 purified monocytes. Furthermore, reduction of the monocyte stimulator-cell concentration demonstrated that the initial concentration of these cells was not suppressive.

Requirements for proliferative vs. cytotoxic responses to hapten-modified cells. Our previous data demonstrated that optimal secondary proliferative responses of human lymphocytes to hapten-modified cells require *HLA-D*-region homology between responder and stimulator cells. On the other hand, Shaw et al. (17, 34) found that T cells primed to TNP-modified cells lysed modified allogeneic targets to the same extent as modified autologous targets, although partial *HLA-A*- and -*B*-locus restriction of cytotoxic responses was demonstrated by cold target-blocking studies. To determine whether the specificities involved in these two types of T cell-mediated responses were, in fact, different, and not readily explained by uncontrolled technical variables between laboratories, we compared proliferative and cytotoxic responses within the same experiment (Fig. 2). Primed responder cells were restimulated with hapten-modified or unmodified autologous cells, allogeneic human cells that did not share any HLA determinants with the responder, or mouse cells. Although the modified allogeneic human cells restimulated only 30% of the proliferative response of modified autologous stimulators (Fig. 2, left panel),

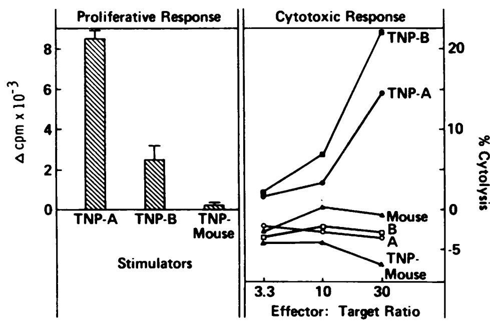

FIGURE 2 Antigenic requirements of proliferative vs. cytotoxic responses to hapten-modified cells. In the left panel the bars represent the net proliferative responses of primed A responder cells restimulated with autologous cells (A), allogeneic human lymphoid cells which did not share HLA antigens (B), or murine splenic lymphocytes. In the right panel the cytotoxic response of primed A responder cells restimulated with hapten-modified A cells was assayed against A, B, or mouse targets which were either TNP-conjugated (closed symbols) or unconjugated (open symbols). Proliferative responses were measured on day 3 and cytotoxic responses on day 4 after secondary stimulation. The standard errors for cytotoxic responses were always <2% of the total releasable radioactivity.

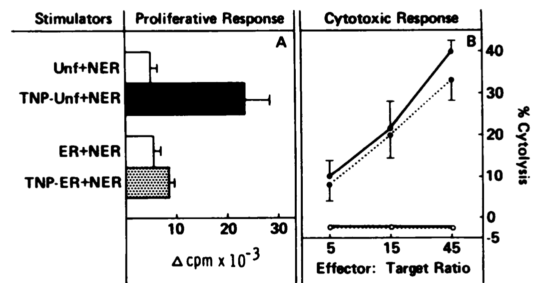

FIGURE 3 Stimulator-cell requirements for cytotoxic responses are different from those for proliferative responses. This figure shows the pooled results of three separate experiments in which responder cells were restimulated with either TNP-conjugated unfractionated (Unf) PBMC or with TNP-conjugated E-rosette (ER) stimulator cells. Non-E-rosette (NER) cells were added to all cultures to control for possible accessory cell requirements. Four days after stimulation, secondary proliferative responses (panel A) and secondary cytotoxic responses (panel B) were assessed. (panel A) The bars represent the mean cpm±SEM; control responses to unmodified cells are shown in the open bars adjacent to the responses to TNP-modified unfractionated (solid bar) or E-rosette (stippled bar) stimulators. (panel B) The ability of effector cells primed with either TNP-unfractionated cells (●) or TNP-E-rosette cells (●) to lyse TNP-conjugated targets (closed circles) or unconjugated targets (open circles) is depicted.

modified allogeneic cells were lysed as well or better than the modified autologous target cells (Fig. 2, right panel). TNP-modified murine cells did not stimulate either proliferative or cytotoxic responses, consistent with the specificity shown by other investigators (34).

Because human cytotoxic responses to hapten-modified cells did not appear to be related to the expression of *HLA-D*-region controlled antigens, we next investigated whether T cells could stimulate cytotoxic responses. Fig. 3 shows the proliferative and cytotoxic responses induced when primed responder cells were restimulated with either hapten-modified unfractionated cells or hapten-modified E-rosette cells. Unmodified non-E-rosette cells were added to all cultures to control for possible macrophage requirements. Recent experiments have shown that macrophages are necessary for accessory function but are not required for hapten presentation in murine TNP-conjugate cytotoxic responses (35). Consistent with the data already presented, modified T cells did not effectively restimulate proliferative responses (Fig. 3A). However, both effector cell populations, those stimulated with modified unfractionated and those with modified T cells, could extensively lyse hapten-modified targets (Fig. 3B). Thus, although T cells could not stimulate proliferative responses, they could induce cytotoxic effector cells.

DISCUSSION

Our data support the concept that some human T cells recognize antigen only in association with autologous non-T-cell lymphoid determinants. Thus, only those cell populations which express easily detectable amounts of *D*-region-encoded Ia-like antigens effectively restimulated proliferative responses of TNP-conjugate-primed T lymphocytes. This finding extends our previous observations of preferential restimulation of autologous TNP-conjugate-primed cells with modified stimulators that share D-region determinants, DRw and Dw (18, 19). A substantial proliferative response, however, was often generated by modified allogeneic stimulators that did not share detected histocompatibility antigens with the primed responder. The stimulatory capacity of modified allogeneic cells which do not share D-region determinants may be a result of extensive cross-reactivity between human histocompatibility antigens or of as yet undetected specificities. Because non-T lymphocytes which function as effective stimulators also express unique antigens encoded outside of HLA the influence of non-MHC antigens in the recognition of hapten must also be considered (36, 37).

The role of macrophages in proliferative responses to TNP-conjugated cells has previously been studied by Thomas et al. (38) in the guinea pig. In accord with the data reported here, these investigators found that TNP-conjugated thymocytes and erythrocytes did not induce proliferative responses; addition of unmodified macrophages to TNP-modified T cells did not restore responses. Similarly, we found that the addition of non-

E-rosette stimulator cells, which contain a high proportion of macrophages (≅50%), had no effect on the net proliferative response to modified T cells. Addition of unmodified T cells to TNP-modified non-E-rosette cells also had no effect. These data militate against the likelihood that macrophage-culture effects, amplifier effects, and suppressor-cell mechanisms explain the failure of modified T cells to induce proliferative responses. Because modified T cells can induce cytotoxic effector cells, these results imply that T cells do not possess structures capable of presenting haptenic determinants to those T cells which respond in proliferative assays.

The histocompatibility requirements for cytotoxic effector-cell function differ from those for proliferative responses. In the human, *HLA-A-* or *-B*-locus homology requirements between killer and target cells have been reported for cytotoxic responses generated against the Y male antigen (13), influenza-infected cells (14, 15), and hapten-modified cells (16, 17). In contrast, we and others have reported the importance of HLA-D-region determinants in the generation of human proliferative responses to haptens and other conventional antigens (18–20, 39–41). In this study we have found that antigen presentation requirements for induction of cells capable of cytotoxic effector function also differ from those for proliferative responses. Nonactivated T cells, which do not express easily detectable amounts of *D*-region-encoded determinants, were capable of inducing secondary TNP-conjugate cytotoxic responses. Thus, the dichotomy between histocompatibility requirements for proliferative and cytotoxic responses is present for response induction as well as at the effector level.

The non-E-rosette population of cells that most effectively presents haptenic determinants is the same PBMC population that stimulates allogeneic mixed leukocyte reactions (42, 43) and autologous mixed leukocyte reactions (43). The observation that glutaraldehyde fixation of TNP-modified cells abrogates the capacity to stimulate secondary proliferative responses is also consistent with previous observations in mixed leukocyte reactions (44). Interestingly, cytotoxic responses to both autologous TNP-modified cells and allogeneic cells can be effectively induced with glutaraldehyde-fixed cells in the mouse (21, 44). Thus the properties of antigenic determinants associated with the induction of proliferative responses differ from those for cytotoxic responses. These results provide further evidence that the distinction between "lymphocyte-defined" or class I molecules (HLA-D) and "serologically defined" or class II molecules (HLA-A and -B), initially demonstrated for reactions to allogeneic cells (45, 46), applies to human immune responses to conventional antigens.

Both adherent monocytes and non-T lymphocytes effectively stimulated secondary TNP-conjugate proliferative responses. The non-T-lymphocyte population contains B cells and nonsurface-immunoglobulin-bearing, non-T lymphocytes (null cells), both of which, in contrast to T cells, express Ia-like determinants (32, 33). Additional studies will be required to ascertain whether B cells or null cells, or both, are capable of effective hapten-conjugate presentation. We found that adherent macrophages restimulated proliferative responses less well than either unfractionated cells or non-E-rosette cells. This finding suggests that it may be possible to distinguish between presentation of antigens by subpopulations of macrophages and other Ia-positive cells for development of proliferative responses. Experiments designed to test this hypothesis are in progress.

Lawrence (47) theorized almost two decades ago that delayed hypersensitivity reactions and autoimmune diseases may be expressions of homograft reactions undertaken by the host against certain of its tissues. For delayed hypersensitivity reactions it now appears likely that antigens are indeed recognized in association with autologous determinants. Proliferative responses of lymphocytes from human subjects sensitized in vivo by dinitrochlorobenzene skin painting are preferentially restimulated in vitro with autologous dinitrophenyl-modified cells (48). Available data suggest that the proliferative responses in vitro are related in magnitude and specificity to delayed hypersensitivity reactions (49–51). Investigations of both proliferative responses to hapten-conjugated cells (18–20) and to purified protein derivative (39–41) suggest that the autologous determinants involved in delayed hypersensitivity reactions are encoded in the *HLA-D* region. Haptenic determinants on soluble proteins or membrane fragments (52) might be presented to T cells by means of adherence to macrophages, transconjugation of haptenic determinants, or macrophage processing, both in vivo and in vitro. Thus it is now reasonable to suggest that in the pathogenesis of delayed hypersensitivity reactions, antigens are presented to host T cells in the context of HLA-D-region determinants. Epidermal Langerhans cells as well as tissue macrophages express Ia-like antigens (53) and thus might also subserve this function. In addition, it seems possible that the association of HLA determinants with a predisposition to certain diseases is intimately related to the antigen presentation role of these determinants in immue responses. Autoimmune disease, cancer, and ageing may all in part be determined by immune responses to subtle alterations in autologous cell surfaces. Further elucidation of T-cell recognitive units and the cellular requirements involved in responses to modified autologous cells

may prove important in understanding the immunopathology of such diseases.

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