HUMAN IMMUNE RESPONSES TO HAPTEN-CONJUGATED CELLS

I. Primary and Secondary Proliferative Responses in Vitro*

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The association between the human major histocompatibility complex (MHC)[1] and a variety of diseases has been well established in the last few years (1, 2). In contrast with the mouse, however, in which the availability of *H-2* recombinant strains has permitted rapid progress, relatively little is known about the functional role of MHC genes in human immune responses. In the mouse, genes within specific regions of the *H-2* complex determine susceptibility to certain diseases (3), control immune responses to numerous antigens (4), and determine proliferative and cytotoxic immune responses of T cells (5–8). Thus, T-cell-mediated cytotoxicity against virus-infected (5) or hapten-modified cells (6) and against minor histocompatibility locus antigens (7) requires syngenicity between target and original stimulator cells with respect to *K* or *D* regions. On the other hand, proliferative responses to protein antigens are facilitated by *I* region homology between interacting macrophages and lymphocytes in both guinea pigs and mice (8, 9). Secondary proliferative responses to hapten-modified cells may also be facilitated by homology within *H-2* (10).

Recently Newman et al. (11) have reported that primed human lymphoid cells respond to hapten-conjugated lymphoid cells in vitro. Using a similar system, the present report describes development of a human model to study both primary and secondary proliferative responses to trinitrophenylated human cells. Moreover, the data connote a role for the human MHC in T-cell recognitive processes. Human lymphocytes primed to hapten-modified autologous peripheral blood mononuclear cells gave substantially greater proliferative responses when restimulated with autologous than with allogeneic hapten-conjugated cells. HLA-A and HLA-B locus antigens do not appear to participate in such restimulation, whereas B-cell typing studies strongly suggest a role for gene products of the HLA-D region in definition of functional immunogenic units.

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[1] *Abbreviations used in this paper:* HBSS, Hanks' balanced salt solution; MHC, major histocompatibility complex; MLC, mixed leukocyte reaction; PBMCs, peripheral blood mononuclear cells; TNP, trinitrophenyl.

Materials and Methods

Peripheral Blood Mononuclear Cells (PBMCs). Heparinized venous blood was obtained from healthy human subjects and PBMCs were isolated by flotation on sodium diatrizoate-Ficoll cushions (Isolymph; Teva, Ltd., Jerusalem, Israel) by previously described techniques (12). Cells were washed twice with Hanks' balanced salt solution (HBSS; Microbiological Associates, Walkersville, Md.) before hapten conjugation or cell culture. Cells obtained in this fashion contain approximately 80% lymphocytes and 20% monocytes. An average of 70% of these cells were classified as T cells on the basis of E-rosetting with sheep erythrocytes.

Trinitrophenyl (TNP)-Conjugated Cells. Hapten-modified PBMCs were prepared by modifications of the technique described by Shearer for murine cells (13). Briefly, $5-25 \times 10^6$ cells/ml were incubated 12 min at 37°C with 2 mM trinitrobenzene sulfonic acid (Nutritional Biochemicals Corp., Cleveland, Ohio) in HBSS buffered with 50 mM Hepes (Microbiological Associates) and titrated to pH 7.8 with 1 N NaOH. Cells were washed three times with Hepes-buffered HBSS supplemented with 10 mg/ml glycylglycine (Grand Island Biological Co., Grand Island, N.Y.) and 5% heat-inactivated fetal calf serum (Grand Island Biological Co.) to remove all unreacted trinitrobenzene sulfonic acid.

Cell Culture Techniques. Cells were cultured in RPMI-1640 (Grand Island Biological Co.) supplemented with 20% AB positive heat-inactivated pooled human plasma, 2 mM L-glutamine (Microbiological Associates) and 50 μg/ml gentamicin (Schering Corp., Kenilworth, N.J.). Cultures were buffered to pH 7.2 with 25 mM Hepes and 1 N NaOH and were incubated in a 5% CO_2 atmosphere at 37°C.

For primary stimulation cells were cultured in either round-bottom microculture wells (1S-MRC-96; Linbro Scientific Inc., Becton, Dickinson & Co., New Haven, Conn.) or in round-bottom 17×100-mm tubes (2001; BioQuest, BBL, & Falcon Products, Cockeysville, Md.). In general, tubes were utilized in long-term cultures for ease in media replacement. Responder and stimulator cells were each cultured at a final concentration of 7.5×10^5 cells/ml in cultures of 0.2 ml (microculture wells) or 2 ml (tubes). For primary sensitization the cells were cocultured for 21–30 days. Primary proliferative responses were monitored from 3 to 25 days after culture initiation. Stimulator cells were treated with 500 or 4,000 rads ^{137}Cs (dose rate 6,000 rads/min) or with mitomycin C (Sigma Chemical Co., St. Louis, Mo., 50 μg/ml for 30 min at 37°C). Inactivated stimulator cells are designated throughout with a subscript x. Culture media were replaced with fresh media once each week.

For secondary restimulation, primed cells were harvested, washed once with medium, and cocultured in microculture wells at a final concentration of 7.5×10^5 viable cells/ml, with an equal concentration of fresh stimulator cells. Stimulator cells in secondary cultures were either TNP-conjugated or unconjugated, and in all experiments were inactivated with 4,000 rads γ-irradiation.

Proliferative responses were assessed by addition of 1.0 μCi of tritiated thymidine (sp act 2.0 Ci/mM; New England Nuclear Corp., Boston, Mass.) to cultures for the final 20 h of the incubation period.

HLA Typing. Typing for HLA-A and HLA-B locus antigens was by standard serologic techniques, employing the microcytotoxicity assay (14). All serologic procedures were performed by personnel of the Methodist Hospital tissue typing laboratory.

B-Cell Antigen Typing. B-cell antigens were detected by using the method of Ting et al. (15). Briefly, a B-cell-enriched population was prepared by rosetting PBMCs with neuraminidase-treated sheep erythrocytes over sodium diatrizoate-Ficoll cushions. The non-erythrocyte rosetting (interface fraction after centrifugation) B-cell-enriched population was then typed for B-cell antigens by using anti-B cell antisera trays kindly provided by Dr. Paul Terasaki, University of California at Los Angeles, utilizing techniques similar to A and B locus typing.

Data Analysis. Data from separate experiments are expressed as mean cpm of three to four replicate cultures with the SEM. Net counts per minute (E-C) were calculated by subtracting cpm of responses to unconjugated stimulators (C) from cpm of cultures with TNP-conjugated stimulator cells (E). E-C errors were determined by the formula for propagation of error. Stimulation indices (E/C) were calculated by dividing (E) by (C) values as defined above. Two-tailed rank-sum tests were performed to determine significant differences between experimental groups.

TABLE I
Primary Responses of Fresh PBMCs to Autologous TNP-Conjugated Cells *

High responders‡	Unconjugated§	TNP-Conjugated	E-C	E-C Range‖
I	798 ± 139	20,813 ± 1,781	20,015 ± 1,951	11,370 – 20,015
Q	2,116 ± 984	17,920 ± 1,768	15,804 ± 2,023	6, 746 – 19,079
A	377 ± 48	7,533 ± 822	7,156 ± 825	6,760 – 15,507
E	587 ± 139	7,306 ± 726	6,718 ± 735	5,842 – 6,718
Low responders				
G	1,310 ± 198	4,520 ± 644	3,210 ± 674	194 – 4,087
N	566 ± 151	2,213 ± 682	1,646 ± 698	1,104 – 1,646
L	493 ± 49	871 ± 319	378 ± 323	187 – 378
K	322 ± 79	997 ± 50	675 ± 105	−472 – 675

* 1.5×10^5 responder cells, cocultured with 1.5×10^5 unconjugated or TNP-conjugated inactivated autologous cells, were pulsed with [³H]thymidine for 20 h 5-6 days after culture in microtiter wells.

‡ High and low responders were distinguished using 2-7 separate experiments for each individual. Each low responder was assayed with a high responder in at least two experiments. High responders in all experiments had E/C values > 5, whereas low responders in all experiments had E/C values < 5.

§ Data are taken from representative experiments with triplicate or quadruplicate determinations.

‖ E-C range of all experiments performed for each subject.

Results

Primary TNP-Conjugate Responses. In vitro coculture of PBMCs with autologous TNP-conjugated cells stimulated primary proliferative responses in 16 of 18 individuals tested. Patterns of relative responsiveness were consistent and clearly differentiated high and low responders (Table I). 5 of 18 subjects tested were classified as low responders in that they always had stimulation indices of less than 5. High responders had an average stimulation index of 11 ± 1 (E/C ± SEM) compared with 2.1 ± 0.3 for low responders. This difference was not due to a shift in response kinetics, nor was it due to a general decrease in response capacity of low responder subjects in that high and low responders exhibited comparable proliferative responses to phytohemagglutinin and delayed skin test reagents (D. L. Peavy and M. F. Seldin, unpublished observations).

Studies of the effect of stimulator cell inactivation on primary proliferative responses to TNP-conjugated cells revealed no difference in the proliferative responses of lymphocytes from high responder subjects to stimulators inactivated with 4,000 rads compared with 500 rads. In contrast, low responder lymphocytes, did not respond to high-dose irradiated stimulator cells, although marginal but significant responses were generated with cells from three out of five low responder subjects when stimulator cells were not inactivated or when they were inactivated with 500 rads or with mitomycin C.

Secondary Responses. Restimulation of primed responder cells 3 wk after initial sensitization generated a secondary proliferative response to TNP-conjugated autologous PBMCs. The kinetics of the primary and secondary responses were easily distinguishable (Fig. 1). Primary responses showed little if any
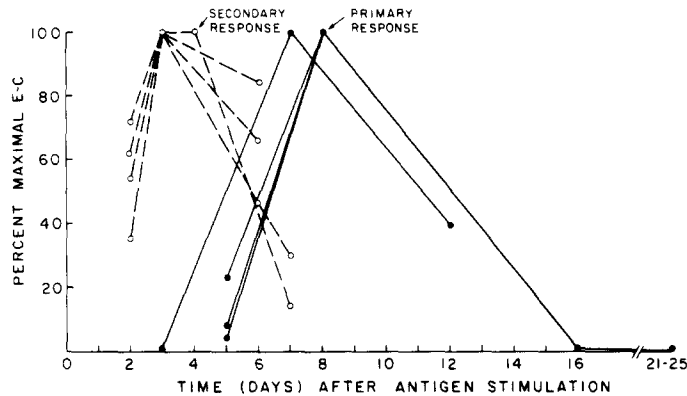
Fig. 1. Kinetics of primary and secondary proliferative responses to hapten-modified autologous cells. Data were taken from three separate experiments and normalized to percent maximal E-C response.

proliferation at day 3 and peaked at day 8; in contrast, secondary responses were maximal at day 3 and had diminished substantially by day 6. The peak secondary response was usually greater than the maximum primary response seen at a later time point. The stimulation index of secondary responses varied from 6.5–20 and averaged 14-fold at day 3. PBMCs precultured for 3 wk without stimulator cells generated responses consistent with primary responses, both in magnitude and kinetics, when TNP-conjugated stimulator cells were added after the preincubation period.

In general, cells from subjects which gave low primary responses failed to give responses after secondary restimulation. Low primary responder G (Table I), however, gave both primary and secondary responses when primary stimulators were mitomycin C-treated (Table II) or not inactivated (Table III), although no secondary response was seen when primary stimulators were treated with 4,000 rads. Secondary responses were not affected by inactivation of secondary stimulators with the high dose (4,000 rads) of γ-irradiation.

Preferential Restimulation with Autologous TNP-Conjugated Cells. To investigate whether secondary TNP-conjugate responses were dependent on recognition of some autologous determinants in addition to the hapten moiety, primed responders were restimulated with both autologous and heterologous TNP-modified PBMCs. The results of one such experiment are seen in Table II. In this experiment a self-preference in the secondary response was seen. That is, the response to autologous TNP-conjugated stimulators was much greater than that to allogeneic TNP-conjugated stimulators. In contrast to data reported in the mouse, in which cells primed with TNP-conjugated autologous cells were unresponsive to alloantigens (10), the response of autologous TNP-conjugate primed cells to alloantigen was often accelerated. Furthermore unprimed precultured cells developed proliferative responses following the usual kinetics of human MLC responses.

Influence of HLA-A and HLA-B Locus Determinants on Secondary TNP-Conjugate Responses. We next considered whether MHC determinants were involved in the recognition of TNP-conjugated cells. Fig. 2 shows a single experiment in which two responders were primed to autologous TNP-conjugated PBMCs and then restimulated with the same panel of three stimulators who

TABLE II
Self-Preference of Secondary Response to Hapten-Modified PBMCs

Stimulators* (HLA Phenotype)				G Responders‡	
A		B		Unprimed	Primed
	G		Unconjugated	227 ± 34	756 ± 69
(1,2		27,40)	TNP-Conjugated	401 ± 71	10,998 ± 719
	H		Unconjugated	1,945 ± 146	5,841 ± 146
(3,W33		15,17)	TNP-Conjugated	1,041 ± 164	5,877 ± 403

* TNP-conjugated or unconjugated stimulators were fresh PBMCs inactivated with 4,000 rads and cultured at 1.5 × 10^5/microtiter well. Cultures were harvested 68 h after secondary stimulation.

‡ Responders were cultured at 1.5 × 10^5 cells/microtiter well. Unprimed responders were fresh PBMCs. Primed responders were cocultured 3 wk with mitomycin C-inactivated trinitrophenylated autologous cells.
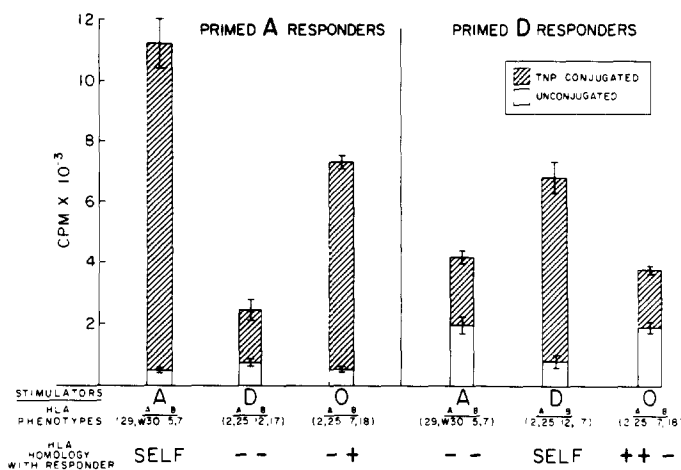

FIG. 2.   HLA association of secondary responses. Primed responders were cocultured with 500 rad inactivated TNP-conjugated autologous cells and restimulated with 4,000 rad inactivated TNP-conjugated or unconjugated cells. Mean cpm ± SEM of responses to conjugated cells are represented by the total height of each bar, with the response to unconjugated cells shown in the clear area. As will be subsequently discussed (see text and Table IV) stimulator O shares a D region antigen (DRw2) with Responder A, in addition to the HLA-B7 antigen.

had been typed for HLA-A and HLA-B locus serologic specificities. For both responders there was a proliferative response to TNP-conjugated allogeneic cells in excess of that to alloantigens alone. The level of proliferation of one responder to TNP-conjugated cells that shared both HLA-A antigens was the same as the response to a completely HLA-nonidentical stimulator. That is, the responses of primed cells from subject D to stimulator cells O and A were essentially equivalent and were about 30% of that to autologous TNP-conjugated cells (E-C analysis). The response of the other responder (A) showed a much higher level of proliferation to allogeneic TNP-conjugated cells that shared an HLA-B antigen (O) than to completely HLA-nonidentical stimulator cells (D).

A further examination of the functional role of HLA-A and HLA-B antigen homologies in secondary TNP-conjugate responses was therefore performed (Table III). The presence or absence of HLA-A antigen homology did not affect

TABLE III
Effect of HLA A and B Locus Homology on Secondary Responses

TNP-Conjugated stimulators (HLA phenotype) A / B	Primed G responder* HLA Homology A-1,2	B-27,40	E-C ± SEM§	Primed M responder‡ HLA Homology A-2,3	B-12,40	E-C ± SEM	Primed O responder HLA Homology A-2,25	B-7,18	E-C ± SEM
$GTNP_x$ (1,2 / 27,40)	Self		15,588 ± 1,975	ND¶		ND¶			ND
$BTNP_x$ (10,W32 / 5,W35)	–	–	361 ± 1,183			ND			ND
$MTNP_x$ (2,3 / 12,40)	+	+	750 ± 865	Self		19,436 ± 2,645	+	–	2,944 ± 255
$OTNP_x$ (2,25 / 7,18)			ND			ND	Self		6,074 ± 258
$JTNP_x$ (1,3 / 5,8)			ND			ND	–	–	2,105 ± 453
$PTNP_x$ (2,W31 / 5,12)			ND	+	+	4,634 ± 641	+	–	213 ± 401
$TTNP_x$ (W24,W31 / 7,14)			ND	–	–	9,181 ± 1,220	–	+	3,873 ± 530
$CTNP_x$ (1,9 / 7,8)			ND			ND	–	+	6,008 ± 372

* G primed responders were from cultures with autologous TNP-conjugated cells that were not inactivated.
‡ M primed and O primed responders were from cultures with autologous 4,000 rad inactivated TNP stimulators.
§ Secondary cultures of all responders were harvested 68 h after culture initiation.

TABLE IV
DRw Typing of Subjects

Subjects	B-Cell antigens*
G	No antigens detected
H	DRw1 (weak reaction)
B	Not typed
M	DRw2, DRw1, DRw6
O	DRw2
J	DRw3
P	DRw7
T	DRw7
C	DRw2, DRw3 (weak reaction)
A	DRw2
D	DRw7, DRw3 (weak reaction)

* 1977 WHO nomenclature for D-region related (DR) antigen (17).

the magnitude of responses, whereas HLA-B antigen homology correlated in some but not all, combinations with higher responses. One stimulator (C) which shared an HLA-B antigen with the primed responder (O) gave a proliferative response equivalent to autologous conjugated cells.

Influence of HLA-D Region Determinants on Secondary TNP-Conjugated Respones. In each experiment in which HLA-B antigen homology between stimulator and responder cells resulted in augmented responses the antigen shared was HLA-B7. Our attention was therefore directed to the *HLA-D* region, since *HLA-B7* is in strong linkage disequilibrium with *Dw2* (16).

Two alternative methods, MLC responses to homozygous typing cells (Dw specificities) and serologic detection of B-cell antigens can be used to determine D region alloantigens. The B-cell antigens utilized in our investigation have been designated DRw antigens, corresponding to the Dw specificities detected by the functional tests (WHO Conference 1977 [17]).

In the two examples where HLA-B antigen homology was clearly associated with a higher response than other allogeneic stimulators (Fig. 2, Responder A with Stimulator O: Table III, Responder O with Stimulator C) the individuals also shared the DRw2 antigen (Table IV). In addition the DRw2 antigen was also shared (Table III, Responder O) with a stimulator (M) that was not as effective as a DRw2 negative subject (T) in restimulating the response. It may be relevant in this regard that subject M was Black, whereas with the other DRw homologies the subjects were Caucasian.

More data relevant to the question of HLA-D region antigen association of the secondary proliferative response are shown in Table V. Stimulators that shared HLA-D region antigens with the responder cells generally gave higher responses, expressed as a percentage of the response with autologous stimulators (76.5 ± 5.3%; mean ± SEM), than did those that did not share these antigens (42.0 ± 7.0%). It should be noted that in experiment 1, stimulator Z was the son of responder A. Thus haploidentity in the one experiment where this was examined was not more effective than HLA-D antigen homology between allogeneic individuals. The data presented in this table also illustrate the finding that the response generated by allogeneic TNP-conjugated cells was

TABLE V
HLA-D Antigen Association of Secondary Proliferative Responses

Stimulator HLA phenotype A	B	Primed A Responders HLA Homology A	B	HLA-D Antigen* homology	% Maximal‡ E-C ± SEM Exp. 1§	Exp. 2	Primed O Responders HLA Homology A	B	HLA-D Antigen homology	% Maximal E-C ± SEM Exp. 1	Exp. 3‖
ATNPx (29,W30	5,7)			Self	100 ± 1	100 ± 8	−	+	+	83 ± 2	
OTNPx (2,25	7,18)	−	+	+	88 ± 2	64 ± 1			Self	100 ± 1	100 ± 4
CTNPx (1,9	7,8)	−	+	+	69 ± 3		−	+	+	71 ± 3	99 ± 6
ZTNPx¶ (1,W30	5)	+	+	+	60 ± 1		−	−	−	53 ± 2	
TTNPx (W24,W3	7,14)	−	+	−	39 ± 3		−	+	−	57 ± 4	64 ± 9
DTNPx (2,25	12,17)	−	−	−	31 ± 1	17 ± 3	++	−	−	44 ± 1	

* Except for stimulator Z (see footnote ¶) the HLA-D antigen homology between responder and stimulator where indicated was in each case the DRw2 antigen (see Table IV), reflecting the strong linkage disequilibrium between HLA-B7 and this D region allele.

‡ Percent maximal E-C was calculated by determining the mean response to stimulators from triplicate or quadruplicate cultures and employing the following formula:

$$\frac{(cpm\ allogeneic\ TNP\text{-}conjugated) - (cpm\ allogeneic\ unconjugated)}{|(cpm\ syngeneic\ TNP\text{-}conjugated) - (cpm\ syngeneic\ unconjugated)|} \times 100\%.$$

§ In experiments 1 + 2 primed responders (A and O) were generated by coculture with 500 rad inactivated autologous trinitrophenylated cells. Secondary cultures were harvested at 48 h.

‖ In experiment 3 primed O responders were generated by coculture with 4,000 rad inactivated autologous trinitrophenylated cells. Secondary responses were harvested at 68 h.

¶ Stimulator Z is the son of Responder A.

not entirely explained on the basis of shared MHC specificities, insofar as they were detected.

HLA-D region determinant homology between primed responder and stimulator correlated significantly with the magnitude of the secondary TNP-conjugate proliferative response. The hypothesis that the two samples (those sharing and not sharing HLA-D antigens) were drawn from the same population was rejected ($P < 0.01$; two-tailed rank-sum test). On the other hand, stimulators with HLA-A or HLA-B homology with the responder (excluding stimulators where HLA-D antigen homology with the responder was also present) were not significantly different from stimulators with no responder homology ($P > 0.2$).

Discussion

This investigation was undertaken to develop an in vitro model for examination of the functional significance of MHC determinants in human immune responses. We studied proliferative responses of human lymphoid cells which had been conjugated to a well-defined hapten (TNP), based on the previous findings of MHC association in murine responses (10). Recently methods for obtaining primed proliferative and cytotoxic responses to hapten-modified human lymphoid cells have been reported (11, 18). Although self-preference was suggested, the role of the MHC, if any, in these responses was not established. Our experiments demonstrate that under appropriate conditions human lymphocytes develop both primary and secondary responses in vitro to TNP-conjugated lymphoid cells. Primary proliferative responses have not been previously reported for either human or murine TNP-conjugate systems.

Based on primary proliferative responses to TNP-conjugated autologous cells, individuals could be segregated into high and low responders. Five of 18 subjects studied were classified as low responders. Three of these low responders generated minimal primary responses but were extremely sensitive to stimulator cell inactivation. A likely explanation for this observation is that functional stimulator cells are required in greater numbers or for a longer time period to trigger responses in these individuals. This in turn may be caused by decreased efficiency in antigen presentation, or recognition, or both.

Although we can only speculate on mechanisms responsible for high and low responsiveness, it is unlikely that previous exposure (priming) to TNP had occurred. Furthermore these same subjects did not segregate into high and low responders when stimulated with mitogens or delayed skin test reagents. Similar phenomena in proliferative responses to soluble antigens (4) and cytotoxic responses to TNP-conjugated cells (6) in the mouse have been explained on the basis of *Ir* genes encoded within the *I* region of *H-2*, which is analogous to the *HLA-D* region. The possibility that high and low responsiveness to TNP-conjugates in humans may reflect presentation or recognition of particular *D* region gene products, rather than responsiveness to TNP per se, is therefore particularly interesting. Responsiveness to other haptens in the same system may resolve this possibility.

A major interest has been the possible involvement of MHC gene products in stimulation of secondary responses. The data clearly demonstrate self-preference in the secondary stimulation phase, although various levels of responsive-

ness to allogeneic TNP-conjugated cells were seen. Several possible mechanisms were considered to explain both self-preference and apparent reactivity to TNP on allogeneic cells in the secondary response. (a) Since alloantigens can stimulate suppressor T-cell function (19, 20) the apparent self-preference of the secondary response may be due to the absence of alloantigen determinants rather than a requirement for autologous structures. We consider this explanation unlikely because of the accelerated kinetics of the secondary response. Furthermore, preliminary experiments demonstrate that the addition of allogeneic cells to secondary cultures does not suppress responses to autologous TNP-conjugated stimulators. (b) Part or all of the response may be to TNP-protein on the cell surface without special regard to HLA. Self-preference may, therefore, be due to autologous proteins having unique determinants. While not formally excluded, this possibility conflicts with the findings in murine models and would not explain our findings of preferential restimulation with partial HLA-D region homologous stimulators. (c) There may be a requirement comparable to that in murine systems for certain MHC determinant homologies. Our data did strongly suggest that D region homology between primed responder and stimulator account for increased responsiveness. It should be stated, however, that the limited number of D region alleles which can be currently detected constrain this conclusion. It is possible, of course, that the requisite genetic homologies between responder and stimulator cells are not with detected alloantigenic determinants, but with other HLA-D region gene products in linkage disequilibrium with the typed alleles. In murine secondary proliferative responses to TNP-conjugated cells a strict MHC homology restriction was seen, but intra-H-2 mapping was complicated (10). $K + I$ region homology was more effective in restimulating secondary responses than I region homology alone. Since all subjects in this study with DRw2 homology also shared the HLA-B7 antigen a similar cooperative interaction between HLA-B and D region determinants must be considered.

It must be emphasized that allogeneic TNP-conjugated cells which did not share any detected MHC determinants with the primed responder still frequently stimulated a measurable proliferative response, albeit substantially less than autologous cells. The role of the human MHC in T-cell-mediated responses to conventional antigens is not yet clear. Differences between apparent mouse and human homology requirements may be explained by more extensive crossreactivities between human than murine MHC determinants (i.e., differences between outbred and inbred populations). Recent work with wild mice has shown that cross-reactivities in the TNP cell-mediated lympholysis assay do occur in H-2 disparate hosts when the H-2 haplotypes between stimulator and target cells are closely related (21). A less likely explanation is the possibility that the recognitive structures in human lymphocytes are less discriminating than murine receptors. That is, greater cross-reactivity at the receptor level rather than the stimulator determinant level could account for disparities.

Alternatively, the apparently nonrestricted component of the response may represent proliferation induced by TNP-conjugated allogeneic proteins presented by syngeneic macrophages. Since syngeneic macrophages are present in

both the primary and secondary phase, MHC restriction requirements would not preclude a proliferative response dependent on macrophage handling of TNP-conjugated proteins on the cell surface. The much lower percentage of macrophage-monocytes in murine spleen cell populations (3-5%) than in human PBMC populations (20-25%) could explain the strict homology requirements in secondary TNP-conjugate proliferative responses reported in the mouse (10).

Recently Dickmeiss et al. (22) reported that cell-mediated cytotoxicity against hapten-conjugated lymphocytes is HLA restricted. They utilized dinitrophenylated PBMCs from subjects who had been contact-sensitized in vivo with dinitrochlorobenzene or dinitrofluorobenzene. After an additional in vitro sensitization phase, the cytotoxic responses were largely restricted to HLA-A or HLA-B locus homologous hapten-conjugated targets. Thus, their findings with cytotoxicity contrast with our results that human proliferative responses to hapten-conjugated cells are associated with the HLA-D region, and both sets of data are in accord with previous studies in mice (6, 10).

The role of HLA determinants in recognition and/or presentation of conventional antigens may help elucidate the pathogenesis of a variety of diseases. The association of a number of human diseases with certain HLA alleles, probably a reflection of linkage disequilibrium, has become apparent in recent years (1, 2). Moreover, juvenile diabetes mellitus (23, 24) and ragweed hayfever (25), which are not associated with particular HLA alleles but segregate with the MHC in family studies, may be caused in part by expression of Ir genes encoded in the D region. We found that secondary proliferative responses of human lymphocytes to TNP-conjugated lymphoid cells are associated with the HLA-D region. Thus the model we describe has important implications for definition of human T cell recognitive units and may permit a functional characterization of the role of HLA determinants in both normal and pathologic states.

Summary

An in vitro model was developed to study both primary and secondary proliferative responses of human lymphocytes to hapten-conjugated peripheral blood mononuclear cells. Coculture of human lymphocytes with autologous trinitrophenyl (TNP)-conjugated stimulator cells resulted in primary proliferative responses. Subjects segregated into high and low primary responders with mean stimulation indices of 11 and 2.1, respectively. Restimulation of primed cells from high responder subjects 3 wk after initial sensitization generated secondary proliferative responses.

To investigate the antigenic requirements for secondary stimulation, autologous TNP-conjugate primed responders were restimulated with both autologous and allogeneic TNP-conjugated stimulators. In all experiments restimulation with autologous conjugated cells yielded substantially greater proliferative responses than with allogeneic conjugates. Experiments were then performed to ascertain whether HLA determinant homology between primed responder and stimulator cells influenced the level of secondary responsiveness. Homology for HLA-A and B locus serologic determinants was not associated with enhanced responsiveness. In contrast, D region determinant homology, detected by B-cell

antigen typing, showed a highly significant positive correlation with the magnitude of secondary responses. The data thus strongly suggest that for secondary proliferative responses to TNP, human T cells recognize hapten in association with HLA-D region determinants.

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