NATURE OF THE ANTIGENIC COMPLEX RECOGNIZED BY T LYMPHOCYTES

I. Analysis with an In Vitro Primary Response to Soluble Protein Antigens

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The major histocompatibility complex (MHC)[1] encodes a number of genes that control or regulate the immune response.

Several genes mapping in the immune response (*I*) region of the MHC appear to control the interaction of immunocompetent cells including the interaction of thymus-derived (T) lymphocytes with bone marrow-derived (B) lymphocytes (1) or macrophages (2, 3). Two concepts have been proposed to explain the histocompatibility restrictions in immunocompetent cell interactions. According to one proposal (the cellular interaction structure model) the *I*-region genes code for specific cellular interaction structures and that homology between these structures is necessary for effective cellular interactions. A second proposal (the complex antigenic determinant model) is based on the observations that mouse T cells sensitized to hapten- or virus-modified cells are primarily cytotoxic for similarly modified target cells which are *H-2D* or *H-2K* compatible (4, 5). These observations suggest that T cells do not recognize antigens per se, but can only be sensitized to antigen-modified membrane components or to complexes of antigen combined with certain membrane molecules.

Previous studies have demonstrated that in the guinea pig effective interaction between antigen-pulsed macrophages and immune T cells required that the macrophages and T cells be syngeneic (2, 6, 7). Because the MHC's of strain 2 and strain 13 animals differ only with respect to *I*-region antigens, it was proposed that identity between *I*-region antigens was required for effective macrophage-T-cell interaction. Identity at the B region of the guinea pig MHC, the homologue of the mouse *H-2K* and *H-2D* regions, was neither sufficient nor necessary for T-cell activation. Although these experiments are consistent with the cellular interaction structure model, it should be noted that these studies were performed with immune T cells. Another explanation for the failure of strain 13 macrophage-associated antigen to activate the immune strain 2 lymphocyte in vitro is that the strain 2 T cell has been primed in vivo only to antigen associated with strain 2 macrophages, and not strain 13 macrophages. Therefore, the failure of interaction between antigen-pulsed allogeneic macrophages and immune T cells is also consistent with the complex antigenic determinant model and in this respect can be regarded as a failure of antigen recognition rather than as a failure of cellular interaction.

In order to further analyze the genetic factors involved in the regulation of macrophage-T-cell interactions, we have developed an assay for the generation of an in vitro primary response to soluble protein antigens in which nonimmune guinea pig T lymphocytes can be primed and subsequently challenged in tissue culture with antigen-pulsed macrophages. Experiments can thereby be performed in which any combination of allogeneic or syngeneic lymphocytes and macrophages can be tested. The results presented in this study are compatible with the proposal that T cells do not respond to native antigen and are primarily sensitized to an antigen-induced modification of the stimulator cell.

Materials and Methods

Animals. Inbred strain 2, strain 13, and (2 × 13)F$_1$ guinea pigs were obtained from the Division of Research Services, National Institutes of Health, Bethesda, Md.

Preparation of Cells. Guinea pigs were injected intraperitoneally with 25 ml of sterile mineral oil (Marcol 52; Humble Oil & Refining Co., Houston, Texas) and the resulting peritoneal exudate was harvested 3–4 days later. This cell population consisting of approximately 75% macrophages, 10% neutrophils, and 15% lymphocytes was used as a source of macrophages for antigen pulsing (see below). A T-lymphocyte-enriched cell population was prepared by passing lymph node cells from animals injected in the foot pads several weeks previously with complete Freund's adjuvant (Difco Laboratories, Detroit, Mich.) over a rayon wool adherence column (8).

In Vitro Antigen Priming. Unfractionated peritoneal exudate cells (5–10 × 10^6/ml) were incubated for 1 h at 37°C in Hanks' balanced salt solution containing 25 μg/ml of mitomycin C (Sigma Chemical Co., St. Louis, Mo.) and 100 μg/ml of ovalbumin (Ova, five times crystallized; Pentex Biochemical, Kankakee, Illinois). The Ova-pulsed macrophages were washed four times to remove the unbound Ova and mitomycin C. In several experiments the macrophages were treated with trinitrobenzene sulfonate (TNBS) according to the method of Shearer et al. (5). The antigen-pulsed or TNBS-treated macrophages (1 × 10^6) and column-passed lymph node cells (5 × 10^6) were maintained in a total vol of 1.5 ml of EHAA medium (9) containing L-glutamine (300 μg/ml), penicillin (100 U/ml), 5-fluorocytosine (5 μg/ml), 2-mercaptoethanol (5 × 10^{-5} M), and 5% heat-inactivated normal guinea pig serum (NGPS). During the first culture the cells were incubated for 1 wk at 37°C in 2% CO$_2$ in air (Fig. 1). On the 3rd day of the first culture the medium was decanted and replaced with 1.5 ml of fresh medium.

In Vitro Assay of DNA Synthesis. The antigen-primed T cells (1–2 × 10^5 per well) recovered from the first culture were restimulated in a second culture in flat-bottom microtiter plates (Cooke Laboratory Products Div., Dynatech Laboratories Inc., Arlington, Va.) with fresh Ova-pulsed peritoneal exudate cells (1 × 10^5 per well) in a total vol of 0.2 ml of EHAA medium containing 5% NGPS (Fig. 1). In some experiments purified antigen-pulsed macrophages were obtained by washing away the nonadherent peritoneal exudate cells after a 1 h incubation in the microtiter wells before adding the Ova-primed T cells. After incubation for 3 days at 37°C in 2% CO$_2$ in air 1 μCi of tritiated thymidine ([^{3}H]TdR, sp act 6.7 Ci/mmol; New England Nuclear, Boston, Mass.) was added to each well. The amount of radioactivity incorporated into cellular DNA was determined after an additional 18 h incubation with the aid of a semiautomated microharvesting device (10). The results of triplicate cultures are expressed as either total counts per minute per culture or as the difference between antigen-stimulated and nonstimulated control cultures (Δ counts per minute). The stimulation index refers to the counts per minute obtained from antigen-stimulated cultures divided by the counts per minute from control cultures.

Results

Kinetics and Specificity of the In Vitro Primary Response. During the course of the initial 7-day culture of strain 13 T cells and strain 13 Ova-pulsed macrophages samples of cells were harvested at daily intervals and the amount of DNA synthesis was determined (Fig. 2). The initial 7-day incubation of strain 13 T cells with Ova-pulsed strain 13 macrophages (Ova-MΦ) in tissue culture

NONIMMUNE DONORS

Harvest of PEC ⟶ Ag Pulsing of PEC

Collect Lymph Nodes

Adherence Column

T-Lymphocyte-Rich
Lymph Node Cells

Lymph Node T Lymphocytes and
Ag-Pulsed PEC Mixed in Culture Tube

Ag-Primed T Lymphocytes
Added to Ag-Pulsed   7 Days
Macrophages

Ag-Pulsed PEC Added
to Microtiter Wells

Nonadherent Cells Removed
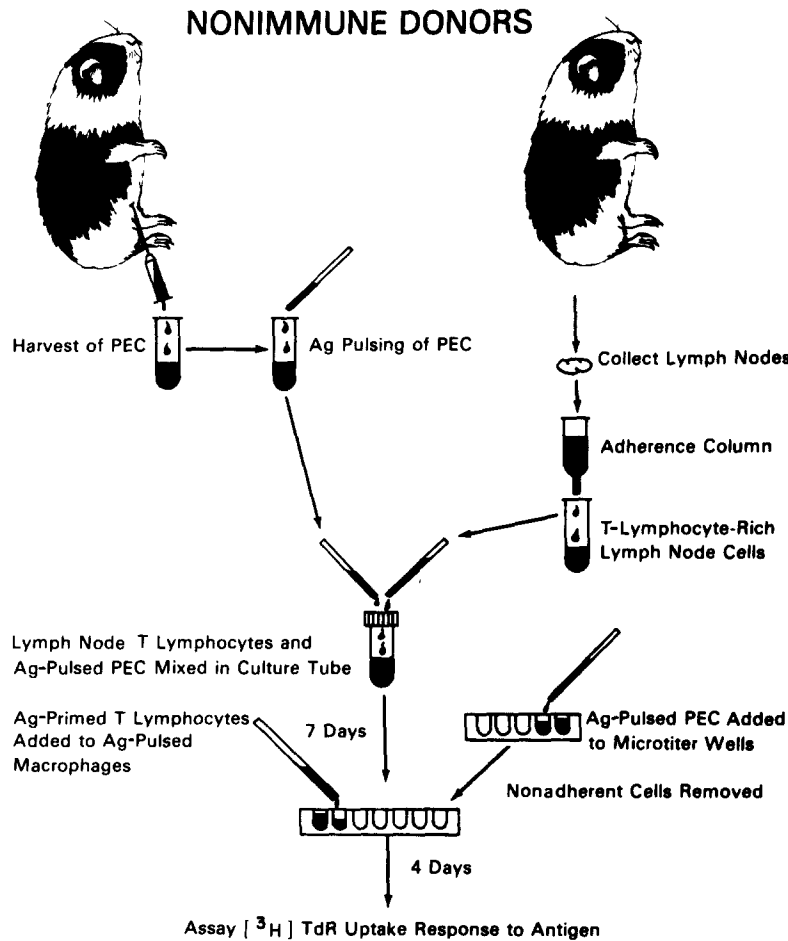
4 Days

Assay [^{3}H] TdR Uptake Response to Antigen

FIG. 1. Schematic diagram for the in vitro priming of guinea pig T lymphocytes with
antigen (Ag)-pulsed macrophages. The details of this procedure are described in the
Materials and Methods. PEC, peritoneal exudate cells.

resulted in only a modest increase in DNA synthesis (two- to threefold) in
comparison to cultures containing nonantigen-pulsed macrophages. After the
first 7 days in culture approximately 20–30% of the original cell number was
recovered; viability was 95% as determined by trypan blue exclusion. When
these cells were transferred to microtiter wells containing fresh Ova-MΦ a
substantial increase in DNA synthesis (60-fold) occurred which was maximal 4
days after transfer. It thus appeared that the T cells had become primed to the
Ova and were capable of giving a substantial secondary response upon rechal-
lenge with fresh Ova-MΦ. The in vitro priming seemed to be specific for Ova and
not a nonspecific activation since Ova-primed T cells failed to respond to human
serum albumin-pulsed macrophages (HSA-MΦ) in the second culture (Fig. 2).
This specificity was more clearly shown in a "checkerboard" experiment in
which separate aliquots of strain 13 T cells were primed with Ova or with DNP
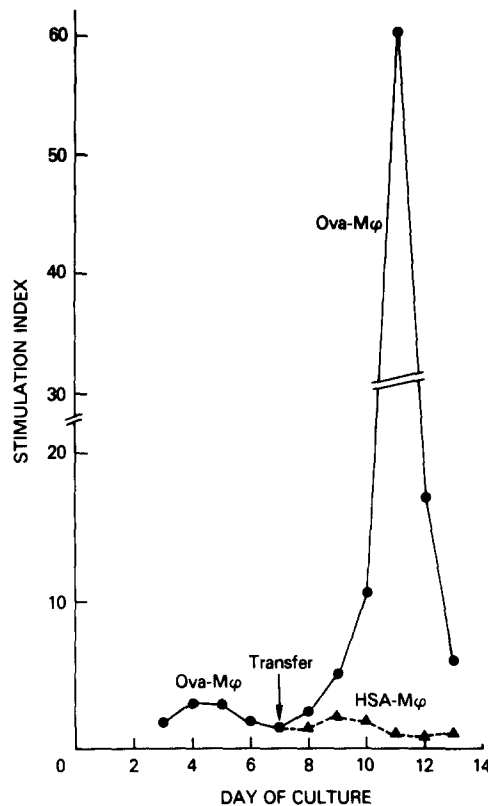guinea pig albumin (DNP-GPA)-pulsed macrophages (Table I). The Ova-primed

Fig. 2.   Kinetics of the primary in vitro response with strain 13 T lymphocytes. Strain 13 T lymphocytes were incubated for 7 days with Ova-pulsed strain 13 macrophages (Ova-MΦ) and transferred to fresh Ova-MΦ in the microtiter wells for an additional 5-day incubation as described in the Materials and Methods. DNA synthesis was determined at daily intervals by the incorporation of [^{3}H]TdR and the results are expressed as the stimulation index of antigen-stimulated to nonstimulated control cultures. Also shown is the response of Ova-primed T cells transferred to microtiter wells containing HSA-pulsed strain 13 macrophages (HSA-MΦ).

T cells could be restimulated with Ova-MΦ, but not with DNP-GPA-pulsed strain 13 macrophages in the second culture. In similar fashion, the DNP-GPA-primed strain 13 T cells could be restimulated in the second culture with DNP-GPA, but not Ova-pulsed macrophages.

Genetic Restriction of the In Vitro Primary Response.   Because we have previously demonstrated genetic restrictions in the in vitro activation of in vivo primed T cells by antigen-pulsed macrophages (2), it was important to determine if similar genetic restrictions in T-cell-macrophage interactions occurred as a result of in vitro priming. We found that if strain 13 T cells were primed with strain 13 Ova-pulsed macrophages they could only be restimulated with Ova-pulsed strain 13, but not strain 2, macrophages in the second culture (Table II). Similarly, strain 2 T cells primed with Ova-pulsed strain 2 macrophages could only be restimulated by strain 2, but not strain 13 Ova-pulsed macrophages (Table III). This genetic restriction on the T-cell response was also obtained when TNBS-treated macrophages were used as the antigen (exp. 3,

TABLE I

Specificity of the In Vitro Primary Response with Strain 13 T Lymphocytes

First culture,* macrophage pulsed with:	Second culture,‡ [³H]TdR cpm		
	Macrophage pulsed with:		
	None	Ova	DNP-GPA
Ova	4,190	<u>58,880</u>	9,050
DNP-GPA	3,630	7,980	<u>16,490</u>

* Strain 13 T lymphocytes were incubated for 7 days with strain 13 macrophages pulsed with 100 μg of Ova or DNP-GPA, as described in the Materials and Methods.

‡ The incorporation of [³H]TdR was determined 4 days after transferring the cells recovered from the first culture to fresh antigen-pulsed strain 13 macrophages in microtiter wells as described in the Materials and Methods. Underlined values indicate cultures in which a positive response has occurred.

TABLE II

Genetic Restriction of the In Vitro Primary Response with Strain 13 T Lymphocytes

	First culture*		Antigen	Second culture,‡ [³H]TdR cpm			
	T lymphocyte	Antigen-pulsed MΦ		2 MΦ		13 MΦ	
				Unpulsed	Pulsed	Unpulsed	Pulsed
Exp. 1	13	13	Ova	3,300	1,800	2,820	<u>103,400</u>
	13	2		223,400	180,500	4,500	4,500
Exp. 2	13	13	Ova	9,130	11,100	10,900	<u>147,200</u>
	13	2		323,900	325,000	5,930	4,670
Exp. 3§	13	13	TNP	31,210	37,960	14,220	<u>83,380</u>
	13	2		215,700	219,900	4,220	13,160

* Strain 13 T lymphocytes were incubated for 7 days with syngeneic or allogeneic Ova-pulsed macrophages as described in the Materials and Methods.
‡ Same as in the footnote for Table I
§ The antigen in this experiment was TNBS-treated macrophages prepared according to the method of Shearer et al (5) instead of Ova.

TABLE III

Genetic Restriction of the In Vitro Primary Response with Strain 2 T Lymphocytes

	First culture*		Antigen	Second culture,‡ [³H]TdR cpm			
	T lymphocytes	Antigen-pulsed MΦ		13 MΦ		2 MΦ	
				Unpulsed	Pulsed	Unpulsed	Pulsed
Exp. 1	2	2	Ova	155	239	1,080	<u>16,000</u>
	2	13		14,240	15,190	728	719
Exp. 2	2	2	Ova	3,820	2,760	2,300	<u>12,550</u>
	2	13		77,800	50,870	3,490	5,700
Exp. 3§	2	2	TNP	20,520	23,750	15,530	<u>77,620</u>
	2	13		60,980	58,070	3,290	11,420

* Strain 2 T lymphocytes were incubated for 7 days with syngeneic or allogeneic Ova-pulsed macrophages as described in the Materials and Methods.
‡ Same as in the footnote for Table I.
§ Same as in the footnote for Table II.

Tables II and III). These results are therefore analogous to the previous findings of Rosenthal and Shevach (2) and suggest that the T cell and macrophage may require similar Ia antigens for efficient interaction. An alternate explanation may be that T cells respond only to the type of antigen-pulsed macrophage with which they were initially sensitized, irrespective of the histocompatibility type of the macrophage.

In order to test these two possibilities, strain 13 or strain 2 T cells were primed with syngeneic or allogeneic strain 2 or strain 13 antigen-pulsed macrophages and restimulated with the respective syngeneic or allogeneic macrophages in the second culture (Tables II and III). It was clear from the results, however, that this approach was not practical since a substantial secondary mixed leukocyte reaction (MLR) was generated with the allogeneic macrophages that obscured any antigen-specific stimulation that might have been present. The T cells were capable of responding to Ova or trinitrophenol (TNP), though, since in the same experiments a good response was elicited by priming with syngeneic antigen-pulsed or TNBS-treated macrophages. The relatively reduced MLR produced by strain 2 T cells responding to strain 13 macrophages, as compared to the 13 against 2 MLR, is a consistent finding as reported previously by Greineder and Rosenthal (11). In the case where the 2 against 13 MLR is relatively weak (exp. 1, Table III) it might have been expected that some antigen-specific stimulation greater than the MLR stimulation alone would have been detected. However, in several experiments the magnitude of the response of strain 2 T cells to either untreated or antigen-pulsed strain 13 macrophages was the same.

Genetic Restriction On the Response of (2 × 13)F₁ T Cells Primed with Antigen-Pulsed Parental Macrophages. In order to circumvent the problem of the MLR we primed (2 × 13)F_1 T cells and subsequently rechallenged them with the strain 2 or strain 13 Ova-pulsed macrophages (Table IV). We found that if the F_1 T cells were initially primed with Ova-pulsed macrophages from one parent they could be restimulated in the second culture only with the Ova-pulsed parental macrophage used for initial sensitization, and not with those of the other parent. For example, F_1 T cells primed with strain 13 Ova-pulsed macrophages responded only to strain 13, but not strain 2 Ova-pulsed macrophages in the second culture. Similarly, F_1 T cells primed with strain 2 Ova-pulsed macrophages were restimulated only by strain 2, but not strain 13, Ova-pulsed macrophages. This genetically restricted response by F_1 T cells was not unique to Ova since the same phenomenon occurred when fetal calf serum-pulsed or TNBS-treated parental macrophages were used as the antigen (exps. 4 and 5, Table IV). In contrast to the genetic restriction observed with parental antigen-pulsed macrophages, F_1 T cells primed with (2 × 13)F_1 antigen-pulsed macrophages could be restimulated in the second culture with either the strain 2 or strain 13 antigen-pulsed macrophages.

Discussion

The technique of an in vitro primary response to soluble protein antigens described in this study provides a method by which the mode of antigen presentation can be easily manipulated. It is thus possible to more clearly define the genetic restrictions on antigen responsiveness by controlling the histocom-

TABLE IV

In Vitro Primary Response of (2 × 13)F₁ T Lymphocytes Stimulated with Antigen-Pulsed Parental Macrophages

First culture*		Antigen	Second culture,‡ [³H]TdR Δ cpm	
			Antigen-pulsed MΦ strain:	
T Lymphocyte	Antigen-pulsed MΦ		2	13
Exp. 1 (2 × 13)F₁	(2 × 13)F₁	Ova	6,750	21,460
	2		2,100	0
	13		10	9,620
Exp. 2 (2 × 13)F₁	(2 × 13)F₁	Ova	27,730	95,680
	2		10,590	530
Exp. 3 (2 × 13)F₁	2	Ova	7,450	980
	13		0	8,200
Exp. 4§ (2 × 13)F₁	2	FCS	11,920	840
	13		0	12,260
Exp. 5‖ (2 × 13)F₁	(2 × 13)F₁	TNP	42,240	11,230
	2		81,220	900
	13		0	27,950

* (2 × 13)F₁ T lymphocytes were incubated for 7 days with (2 × 13)F₁, strain 2, or strain 13 antigen-pulsed macrophages as described in the Materials and Methods.

‡ Same as in the footnote for Table I except that the unstimulated control counts per minute have been subtracted and only the Δ counts per minute are shown.

§ Macrophages were pulsed with fetal calf serum (FCS) as the antigen in this experiment instead of Ova.

‖ The antigen in this experiment was TNBS-treated macrophages prepared according to the method of Shearer et al. (5) instead of Ova.

patibility type of the stimulator cell used to initially present an antigen. In agreement with the previous findings of Rosenthal and Shevach (2) using T cells immunized in vivo, we found that T cells primed with syngeneic antigen-pulsed macrophages in vitro could be restimulated only with syngeneic, but not allogeneic, antigen-pulsed macrophages. These results suggest that either identity is required between the strain 2 or strain 13 *I*-region products for efficient T-cell-macrophage interaction, or alternatively, that T cells respond only to antigen associated with the type of macrophage used for initial sensitization. A direct test of these possibilities by sensitizing T cells with antigen-pulsed allogeneic macrophages was not feasible because of the magnitude of the secondary MLR elicited by the allogeneic stimulus. However when we eliminated the MLR by using combinations of parental macrophages and (2 × 13)F₁ T cells, we found that F₁ T cells primed with antigen-pulsed macrophages from one parent could be restimulated only with the parental macrophage used for initial sensitization, and not with those of the other parent. If only *I*-region identity was required for efficient T-cell-macrophage interaction, then (2 × 13)F₁ T cells, which express both the strain 2 and strain 13 *I*-region antigens, primed with one

parental antigen-pulsed macrophage would have been restimulated with antigen-pulsed macrophages from either parent. In contrast, our results suggest that the primary restriction on the $(2 \times 13)F_1$ T-cell response may be imposed by the type of macrophage used for initial sensitization, irrespective of the I-region antigen which that macrophage expressed.

Based on our results it is likely that some $(2 \times 13)F_1$ T cells are able to be sensitized to the antigenic complex containing strain 2 I-region products while other T cells are sensitized to the immunogenic complex containing strain 13 I-region products. One prediction of this proposal is that in an immunized F_1 animal separate T-cell subpopulations would exist which have been sensitized to antigen associated with either the strain 13 or strain 2 I-region gene products presented by the $(2 \times 13)F_1$ macrophage. In fact, using cells from immune $(2 \times 13)F_1$ donors direct evidence has been obtained for the existence of two populations of lymphocytes, one specific for antigen presented by strain 2 macrophages and the other for antigen associated with strain 13 macrophages (12). In these experiments T lymphocytes from F_1 donors immunized with Ova were positively selected by culturing them for 1 wk with either strain 2 or strain 13 Ova-pulsed macrophages. After positive selection in the primary culture the F_1 T cells showed significant responses when recultured with Ova-pulsed macrophages of the histocompatibility type used in the initial culture, but little or no response to Ova-pulsed macrophages of the other histocompatibility type.

Although the genetic restriction on the response of $(2 \times 13)F_1$ T cells stimulated with parental macrophages supports the complex antigenic determinant concept of T-cell recognition, the cellular interaction structure proposal cannot be ruled out since the F_1 T-cell and parental macrophages share Ia specificities. However, in order for our experimental results to be consistent with the cellular interaction structure model one must postulate allelic exclusion for cellular interaction structures at the T-cell level. We have been unable to demonstrate allelic exclusion for I-region antigens and 95–98% of $(2 \times 13)F_1$ T cells can be stained by either anti-2 or anti-13 serum using indirect immunofluorescence (E. Shevach, I. Green, and W. E. Paul, unpublished observations).

It is also difficult to reconcile the cellular interaction structure model of immunocompetent cell interaction with a number of recent experimental observations which have demonstrated cellular cooperation across an allogeneic barrier. Pfizenmaier et al. (13) have recently demonstrated that H-2 homology between effector and stimulator cells was not obligatory. Thus, when cytotoxic T cells from F_1 radiation chimeras repopulated with lymphocytes from one parent were primed with viral-infected or hapten-modified stimulator cells of the other parental haplotype they could only kill similarly treated target cells expressing the same H-2 antigens as those present during the initial sensitization. Similarly, Pierce et al. (14) found that T-cell helper activity for antibody production from mice immunized with antigen-pulsed allogeneic macrophages could be elicited in vitro only with the same macrophage type as used for the in vivo immunization. Miller et al. (15) have analogous observations for the transfer of delayed hypersensitivity in the mouse. Although we could not directly test the ability of antigen-pulsed allogeneic macrophages to prime across the histocompatibility barrier because of the magnitude of the secondary MLR, under conditions where the MLR has been reduced by anti-Ia sera directed toward the

stimulatory macrophage we could directly demonstrate that T cells can be specifically sensitized to antigens associated with allogeneic macrophages.[2] All of these results strongly suggest that T cells recognize antigen in association with MHC gene products but that MHC identity is not required for efficient immunocompetent cellular interaction.

Whereas mouse cytotoxic T cells primarily recognize antigen associated with *H-2K* or *H-2D* gene products (4, 5), our findings in the guinea pig indicate that the genetic restriction on T-cell activation as measured by proliferative assays is imposed by *I*-region antigens rather than by gene products homologous to the *H-2K* or *H-2D* regions in the mouse. Taken together, these results suggest the possibility that *H-2K*, *H-2D*, or *I*-region genes may serve a similar function in T-cell recognition. That is, either type of gene product may be included in the antigen-induced modification of self-components to create the immunogenic complex. However, T-cell subpopulations perhaps expressing different functions may exhibit a preference for the type of MHC gene product which is included in the complex. It is of interest that in our hands the proliferative response to TNP-modified macrophages or macrophages pulsed with soluble protein both appear to be exclusively under *I*-region antigen regulation. In contrast, Miller et al. (15) have recently demonstrated that *I*-region identity, but not *K* or *D* identity, was sufficient to demonstrate the transfer of delayed hypersensitivity in the mouse when fowl gamma globulin was used as an antigen, while either *I*, *K*, or *D* identity would permit the transfer of delayed hypersensitivity to dinitrofluorobenzene. The discrepancy in these findings may be explained by the different animal species and experimental techniques utilized.

According to the complex antigenic determinant hypothesis, T cells derived from an immunized F_1 animal should be equally sensitized with the antigen-altered Ia complex of both parental haplotypes and should be able to be stimulated in vitro by antigen-pulsed macrophages from either parent. One difficulty with this proposal is the finding by Shevach (7) that for responses controlled by *Ir* genes immune (responder × nonresponder)F_1 T cells could not be activated by antigen-pulsed nonresponder macrophages. One explanation for this finding would be to postulate that the nonresponder macrophage lacks the Ia antigen which can be altered by the antigen under study. If this is the case, then one might predict that nonresponder T cells may be able to be sensitized to antigen-pulsed responder macrophages expressing the necessary Ia antigen. Experiments are now in progress to examine this possibility.

The results we have presented in this study support the concept that T cells are incapable of being activated by antigen per se. Rather, the immunogenic complex seems to involve an antigen-induced modification of self-components which, in the guinea pig, probably includes *I*-region gene products. The exact composition of this immunogenic complex is unknown, but there are several possible roles for the function of the *I*-region products. One possibility is that antigen directly binds to the Ia molecule which modifies the antigen for presentation to the T cell. Alternatively, by binding to the *I*-region gene product the

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[2] Thomas, D. W., and E. Shevach. Nature of the antigenic complex recognized by T lymphocytes. II. T lymphocyte activation by direct modification of macrophage histocompatibility antigens. Manuscript in preparation.

antigen may induce a modification of the Ia molecule which T cells recognize as an altered self-component. Another possibility is that the combination of antigen with *I*-region gene products creates a new complex antigen which contains determinants derived from both the Ia molecule and antigen.

Summary

In order to analyze the genetic factors involved in the regulation of macrophage-T-cell interaction we have developed an in vitro primary response to soluble protein antigens in which nonimmune guinea pig T cells can be sensitized and subsequently challenged in tissue culture with antigen-pulsed macrophages. Antigen-specific T-cell activation, as measured by increased DNA synthesis, occurred when syngeneic antigen-pulsed macrophages were used for both initial sensitization and secondary challenge. No T-cell activation occurred when allogeneic antigen-pulsed macrophages were used for secondary challenge of cells primed with syngeneic macrophages. When allogeneic antigen-pulsed macrophages were used in both primary and secondary cultures it was difficult to assess antigen-specific stimulation due to the substantial mixed leukocyte reaction. However, when T cells from F_1 animals were primed with parental antigen-pulsed macrophages they responded only to the parental macrophages used for initial sensitization but not to those of the other parent. These results are discussed with respect to T-cell recognition of a complex antigenic determinant which may include *I*-region gene products.

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