The Journal of Immunology
RESEARCH ARTICLE | APRIL 01 1980

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J Immunol (1980) 124 (4): 1773–1778.
https://doi.org/10.4049/jimmunol.124.4.1773

0022-1767/80/1244-1773$02.00/0
THE JOURNAL OF IMMUNOLOGY

Vol. 124, No. 4, April 1980
Printed in U.S.A.

THE SYNGENEIC MIXED LEUKOCYTE REACTION: THE GENETIC REQUIREMENTS FOR THE RECOGNITION OF SELF RESEMBLE THE REQUIREMENTS FOR THE RECOGNITION OF ANTIGEN IN ASSOCIATION WITH SELF

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We have used cells from inbred strain 2 and strain 13 guinea pigs in order to define further the role of Ia antigens in the syngeneic mixed leukocyte reaction (MLR). The guinea pig syngeneic MLR resembled the autologous MLR in man in that it demonstrated both memory and specificity. The Ia antigens appeared to be the proliferative stimuli in that the primary stimulator cell was an Ia-positive adherent peritoneal exudate cell (PEC) and the reaction could be specifically inhibited by anti-Ia sera directed to the stimulator cell. We also demonstrated the existence of two (2 × 13)F_1 T cell populations that were capable of reacting to one or the other parental PEC in the absence of any known exogenous antigen. These results suggest that the syngeneic MLR may represent T cell activation mediated through a receptor for self Ia.

It has been known for several years that a proliferative response can be observed when T cells are co-cultured with autologous or syngeneic non-T cells (1–4). Although the autologous mixed leukocyte reaction (MLR) was initially described in studies of the reactivity of neonatal murine thymocytes and syngeneic adult spleen cells (1), more recent investigations in this area have been performed primarily with human cells (5, 6). These studies have demonstrated that the autologous MLR exhibits both memory and specificity (7) and preliminary studies have suggested that the products of the HLA-D region of the human major histocompatibility complex (MHC)[3] may be the relevant target antigens (8, 9). The functional significance of the responding T cell in the autologous MLR is far from clear. Indeed, one recent study has demonstrated that the reactive cell may exhibit T helper cell function (8) whereas others have demonstrated that the responding cell has properties of a T suppressor cell (10, 11).

Received for publication November 7, 1979.
Accepted for publication December 14, 1979.
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[3] Abbreviations used in this paper: BUdR, bromodeoxyuridine; LNL, column purified lymph node lymphocytes; MHC, major histocompatibility complex; NGPS, normal guinea pig serum; PEC, peritoneal exudate cell.

In order to define further the role of Ia antigens in the autologous MLR, we have begun to characterize the syngeneic MLR by using cells from inbred strain 2 and strain 13 guinea pigs whose MHC differ only in the I-region (12). In this report we will demonstrate that the primary stimulator cell of the syngeneic MLR in the guinea pig is an Ia-positive adherent peritoneal exudate cell (PEC), that the reaction can be specifically inhibited by anti-Ia sera directed to the stimulator cell, and that two populations of (2 × 13)F_1 T cells exist that are capable of reacting to one or the other parental PEC in the absence of a known exogenous antigen. Thus, the genetic mechanisms involved in the syngeneic MLR resemble the genetic mechanisms controlling the reaction of guinea pig T cells with antigen-pulsed stimulator cells (13, 14). The implications of these findings in terms of dual recognition models of T cell antigen recognition are discussed.

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.

Immunization of guinea pigs. Guinea pigs were immunized with a 1:1 emulsion of saline in complete Freund's adjuvant (CFA, containing 0.4 mg/ml *Mycobacterium tuberculosis* H_{37} Ra, Difco Laboratories, Detroit, Mich.) injected into the four footpads.

Preparation of lymphocytes. Lymph node cells (500 × 10^6) obtained from animals immunized with CFA were applied to a nylon column containing 3 g of acid washed nylon wool (Fenwal laboratories, Division of Travenal Laboratories, Morton Grove, Ill.) in medium RPMI 1640 (GIBCO, Grand Island, N. Y.) containing 10% normal guinea pig serum (NGPS). After incubation of the cells on the column for 1 hr at 37°C, nonadherent cells were eluted dropwise over a 45-min period. The eluted population contained less than 2% of cells bearing surface immunoglobulin as detected by direct immunofluorescent staining and less than 1% of cells capable of phagocytizing latex beads. This population, which we will refer to as LNL, was used as a source of T lymphocytes.

Preparation of macrophages. Normal unimmunized guinea pigs were injected i.p. with 25 ml sterile mineral oil (Marcol 52, Humble Oil and Refining Co., Houston, Tex.) and the resulting peritoneal exudate was harvested 3 to 4 days later. This cell population (PEC) consisted of 75% macrophages, 10% neutrophils, and 15% lymphocytes and was used as a source of macrophages. In certain experiments adherent macrophages were prepared by incubating the PEC (10 × 10^6/ml) in medium

RPMI 1640 supplemented with 10% NGPS on plastic Petri dishes (Falcon 3002, Falcon, Oxnard, Calif.) for 2 hr at 37°C. The dishes were then washed four times with warm medium to remove nonadherent cells. The adherent macrophages were removed with the aid of a rubber policeman. Ninety to 95% of these cells phagocytized latex beads and >95% appeared to be typical macrophages on light microscopy. Splenic adherent cells were prepared in a similar manner. PEC or purified macrophages (10×10^6/ml) were treated with 25 μg/ml mitomycin C (Sigma Chemical Co., St. Louis, Mo.) for 1 hr at 37°C, washed four times, and then mixed with T lymphocytes.

In vitro assay of DNA synthesis. T lymphocytes (1 to 2×10^6/ml for primary culture and 0.5 to 1.0×10^6/ml for secondary culture) were mixed with mitomycin C-treated PEC (1×10^6/ml) in medium RPMI 1640 containing L-glutamine (300 μg/ml), gentamycin (50 μg/ml, Schering Corp., Kenilworth, N. J.), 2-mercaptoethanol (5×10^{-5} M), and 5% NGPS. Two-tenths milliliter of this cell suspension was cultured in round-bottom microtiter plates (Cooke Engineering, Arlington, Va.) for 4 to 8 days at 37°C in a humidified atmosphere of 5% CO_2 in air. Eighteen hours before harvesting, 1.0 μCi of tritiated thymidine ([^{3}H]-TdR, specific activity 6.7 Ci/mM, New England Nuclear Corp., Boston, Mass.) was added to each well and the amount of radioactivity incorporated into DNA was measured (15). The mean of triplicate cultures is expressed as total cpm per well. The standard error of the mean was never greater than 10% of the mean and for simplicity we have reported only the mean of triplicate samples.

Positive selection cultures. T lymphocytes (5×10^6) were cultured with mitomycin C-treated PEC (2.5×10^6) in a total volume of 3.0 ml of supplemented medium in a 15-ml plastic tube (Falcon 2057) for 10 to 14 days. On the 7th day of culture the medium was decanted and replaced with 3 ml of fresh medium.

Preparation of anti-Ia sera. A strain 13 anti-strain 2 serum (anti-Ia.2,4) and a strain 2 anti-strain 13 serum (anti-Ia.1,3,7) were prepared as previously described (16).

Treatment of T lymphocytes and macrophages with anti-Ia serum and complement (C). Lymphocytes or PEC (10×10^6/ml) were incubated in 1.0 ml of RPMI 1640 in the presence of either NGPS or anti-Ia serum and guinea pig C (GIBCO). The anti-Ia serum was used at a final dilution of 1/10 and a 1/4 dilution of C was used. After a 30-min incubation at 37°C, the cells were washed three times and the cell density was adjusted to an equivalent number of viable cells/ml before addition to culture.

RESULTS

Kinetics of the syngeneic MLR. Initially, we established the conditions for the syngeneic MLR by using cells from inbred strain 2 and strain 13 guinea pigs. Highly enriched populations of T lymphocytes were cultured with syngeneic PEC that has been treated with mitomycin C. A strong proliferative response was observed (Fig. 1) on day 8 of the culture, which gradually declined by day 14. No significant uptake (<200 cpm/well) was observed when T lymphocytes or PEC were cultured alone. After 14 days of culture, cells from multiple microtiter wells were pooled and restimulated with fresh mitomycin C-treated syngeneic PEC. An enhanced proliferative response was observed, which peaked after 4 days of culture.

The syngeneic MLR exhibits memory and specificity. In order to evaluate further the specificity of the syngeneic MLR we used a protocol similar to that described by Weksler and Kozak (7) in studies of the human autologous MLR. T lymphocytes were cultured in tubes with syngeneic or allogeneic PEC for 14 days and then restimulated for 4 days in microtiter wells with syngeneic or allogeneic PEC (Table I). When strain 2 T cells were cultured with strain 2 PEC for 14 days, the recovered cells demonstrated a markedly enhanced secondary proliferative response to syngeneic strain 2 PEC compared to allogeneic strain 13 PEC; strain 2 T cells that had been first cultured with allogeneic strain 13 PEC responded to strain 13 PEC in the second culture, but not to strain 2 PEC. A similar result was observed when strain 13 T lymphocytes were used as responders. However, strain 13 T cells cultured for 14 days with syngeneic PEC still maintained significant reactivity to allogeneic strain 2 PEC in the second culture. The secondary response to syngeneic PEC was still higher than the response to allogeneic PEC (Table I, line 3).

The stimulator cell in the syngeneic MLR is an Ia-positive adherent PEC. It is generally agreed that the stimulator cell in the human autologous MLR is a non-T cell (5, 6). However, considerable controversy exists on the further characterization of this cell (17). We therefore examined the stimulatory capacity of various mitomycin C-treated guinea pig lymphoid populations (Table II). PEC, adherent PEC, and adherent spleen cells

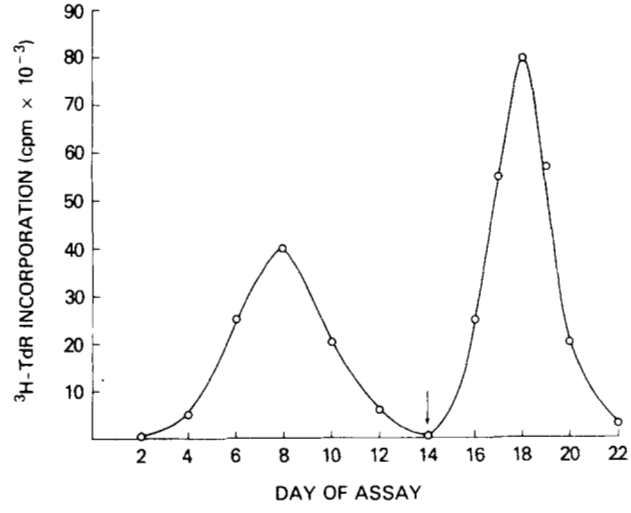

Figure 1. Kinetics of the syngeneic MLR. Strain 2 LNL (0.2×10^6) were cultured with syngeneic PEC (0.1×10^6). The incorporation of [^{3}H]-TdR was assayed on each day indicated. On day 14 the cultured cells were pooled, washed, and 0.1×10^6 viable cells were restimulated with fresh syngeneic PEC (0.1×10^6). The results are expressed as cpm $\times 10^{-3}$/well; each value is the mean of three determinations. An arrow indicates the beginning of the secondary cultures.

TABLE I

Specificity of the syngeneic MLR[a]

First Culture		Second Culture Stimulator	
Responder LNL	Stimulator PEC	2 PEC	13 PEC
2	2	89,993[b]	7,730
2	13	293	63,140
13	13	51,584	104,247
13	2	310,279	1,994

[a] LNL (5×10^6) were cultured with syngeneic or allogeneic PEC (2.5×10^6) for 14 days. Cells recovered from these cultures (0.1×10^6) were then stimulated with fresh syngeneic or allogeneic PEC (0.1×10^6). The incorporation of [^{3}H]-TdR was determined on day 4 of the second culture.

[b] Results are expressed as cpm/well; each value is the mean of three determinations.

TABLE II

Source of the stimulator cell in the syngeneic MLR[a]

Stimulator Cell	[^{3}H]-TdR Incorporation		
	Expt. I	Expt. II	Expt. III
PEC	11,609[b]	11,253	7,591
Adherent PEC	19,339	7,787	11,679
Spleen	339	1,309	1,136
Adherent spleen	N.D.[c]	N.D.	6,412
LNL	371	290	167
Unseparated lymph node	238	1,402	N.D.
Thymocytes	257	150	88

[a] Strain 13 LNL (0.1×10^6) were cultured with syngeneic stimulator cells (0.1×10^6); the incorporation of [^{3}H]-TdR was determined on day 8 of the culture.

[b] cpm/Well; each value is the mean of three determinations.

[c] Not done.

TABLE III

Effect of anti-Ia serum and C treatment on the stimulator cell in the syngeneic MLR[a]

Responder Cells LNL	Stimulator Cells PEC	Treatment	[^{3}H]-TdR Incorporation	
			Expt. I	Expt. II
2	2	NGPS + C	19,781[b]	5,933
2	2	Anti-Ia.2,4 + C	988	892
2	2	Anti-Ia.2,4	12,120	2,449
13	13	NGPS + C	17,077	6,505
13	13	Anti-Ia.1,3,7 + C	1,565	609
13	13	Anti-Ia.1,3,7	10,289	6,051

[a] PEC (10×10^6) were treated with NGPS or anti-Ia serum (final dilution 1/10) in the presence or absence of guinea pig C (final dilution 1/4). For assay of the syngeneic MLR, 0.1×10^6 viable treated PEC were mixed with 0.1×10^6 LNL. The incorporation of [^{3}H]-TdR was determined on day 8 of the culture.

[b] cpm/Well; each value is the mean of three determinations.

were capable of activating the syngeneic MLR. Enriched populations of T cells (thymocytes, LNL) were poor stimulators. Surprisingly, both unfractionated spleen cells and lymph node cells were poor sources of stimulator cells. Since the latter two populations contain a high proportion of B lymphocytes (30 to 50% by immunofluorescent staining), it is unlikely that normal guinea pig B lymphocytes can act as stimulators in the syngeneic MLR.

The results of these studies suggest that the stimulator cell of the syngeneic MLR has certain characteristics in common with the stimulator cells responsible for alloantigen and antigen-induced stimulation of guinea pig T lymphocytes (18). It is likely that this latter cell belongs to the monocyte-macrophage lineage. However, we have previously demonstrated that only 15 to 25% of purified oil-induced guinea pig macrophages can be lysed by treatment with anti-Ia serum and C and that the "Ia-negative" macrophage population is markedly deficient in its ability to present protein antigen to immune T lymphocytes and to function as stimulator cells when mixed with allogeneic T cells in the MLR (19). In order to evaluate the role of Ia antigens in the syngeneic MLR, PEC from both strains were treated with the appropriate anti-Ia serum in the presence of C, washed, and then cultured with syngeneic T cells (Table III). Treatment with anti-Ia and C resulted in lysis of 20 to 25% of the PEC population as determined by trypan blue dye exclusion. However, those cells remaining viable were markedly deficient in their ability to stimulate the syngeneic MLR. In certain experiments a lower degree of suppression was observed when the PEC were treated with anti-Ia serum in the absence of C. This suppression may result from carryover of anti-Ia serum into the culture as the continuous presence of anti-Ia serum in the absence of C also inhibited the syngeneic MLR (see below).

It was also of importance to evaluate the effect of anti-Ia serum and C treatment on the responding T cell population since our previous studies had shown that certain guinea pig T cell functions (the antigen-specific proliferative response of T cells primed *in vivo*) could be dramatically reduced by treatment of the T cells with anti-Ia serum and C whereas other T cell functions (T helper cell function, the ability to respond in the MLR) were unaffected by similar treatment (20). The responding T cell population was therefore treated with the appropriate anti-Ia serum and C (Table IV). Thirty to 40% of the T cells were killed by this treatment, yet the response of the surviving viable cells in the syngeneic MLR was unaffected (strain 13 LNL) or enhanced (strain 2 LNL). The response to allogeneic stimulation was also unaffected by this treatment.

Genetic restriction on the response of (2 × 13)F_1 T cells primed with parental PEC. Previous studies using antigen-specific T cell proliferation or helper activity as a measure of T cell activation have demonstrated two distinct populations of $(2 \times 13)F_1$ T cells that are capable of interacting with antigen associated with macrophages from one or the other parental strains (13, 14, 21). It was of interest, therefore, to examine if two such populations of $(2 \times 13)F_1$ T cells also exist that are reactive with parental PEC in the syngeneic MLR. $(2 \times 13)F_1$ T cells were cultured with either F_1 or parental macrophages for 14 days. Cells recovered from these cultures were then restimulated for 4 days with parental or F_1 PEC. The results of four representative experiments are shown in Table V. F_1 T cells positively selected with PEC of one parent demonstrated an enhanced proliferative response in the second culture to PEC of that parent compared to PEC of the other parent. F_1 T cells selected with F_1 PEC responded in the second culture to PEC of either parental strain as well as to F_1 PEC.

These results suggest that as in the case of antigen-induced T cell proliferation, two populations of $(2 \times 13)F_1$ T cells responsive to one or the other parental PEC also exist in the syngeneic MLR. Since the Ia antigens have been previously shown to be the critical factors in determining the collaborative potential of antigen-pulsed PEC and primed T lymphocytes (22), it was important to determine the role of Ia antigens in the interaction of F_1 T cells and parental PEC in the absence of exogenous antigen. F_1 T cells were positively selected with

TABLE IV

Treatment of the responder cell in the syngeneic MLR with anti-Ia serum and C[a]

Responder Cells LNL	Treatment	[^{3}H]-TdR Incorporation Stimulator Cell	
		2 PEC	13 PEC
2	NGPS + C	11,897[b]	32,189
2	Anti-Ia.2,4 + C	49,945	55,626
2	Anti-Ia.2,4	17,647	28,181
13	NGPS + C	135,444	43,609
13	Anti-Ia.1,3,7 + C	127,494	41,207
13	Anti-Ia.1,3,7	194,910	42,078

[a] LNL (10×10^6) were treated with NGPS or anti-Ia serum (final dilution 1/10) in the presence or absence of guinea pig C (final dilution 1/4). For assay of the syngeneic MLR, 0.1×10^6 viable treated cells were then cultured with 0.1×10^6 syngeneic or allogeneic PEC. The incorporation of [^{3}H]-TdR was determined on day 8 of the culture.

[b] Cpm/well; each value is the mean of three determinations.

parental PEC for 14 days. The recovered cells were then challenged in secondary cultures with F_1 or parental PEC in the continuous presence of NGPS or anti-Ia serum in the absence of C (Fig. 2). As was shown in Table V, F_1 T cells selected with strain 2 PEC responded to F_1 or strain 2 PEC, but not to strain 13 PEC, in the second culture in the presence of NGPS. When the second culture was performed in the presence of anti-Ia.2,4 serum the response to both strain 2 and F_1 PEC was markedly inhibited. The inhibition by anti-Ia serum was highly specific for strain 2 Ia antigens as anti-Ia.1,3,7 serum had no effect on the response of these positively selected F_1 T cells to F_1 PEC. Similarly, the secondary response of F_1 T cells positively selected with strain 13 PEC to F_1 PEC was specifically inhibited by anti-Ia.1,3,7 serum but not by anti-Ia.2,4 serum. These results are therefore consistent with the view that the Ia antigens are the proliferative stimuli of the syngeneic MLR.

TABLE V

Positive selection of (2 × 13)F_1 LNL reactive with F_1 or parental PEC[a]

First Culture (2 × 13)F_1 LNL Cultured with		Second Culture Stimulator		
Expt. no.	PEC	2 PEC	13 PEC	F_1 PEC
I	2	100,534[b]	7,862	46,393
	13	12,656	32,278	72,368
	F_1	122,912	57,344	66,937
II	2	94,199	18,059	77,799
	13	8,567	23,405	39,989
	F_1	23,371	11,161	32,515
III	2	34,821	2,719	28,146
	13	3,523	8,681	17,131
IV	2	12,101	1,352	17,521
	13	2,954	21,401	16,606

[a] (2 × 13)F_1 LNL (5 × 10^6) were cultured with strain 2, 13, or (2 × 13)F_1 PEC (2.5 × 10^6) for 14 days. Cells recovered from these cultures (0.1 × 10^6) were restimulated with fresh strain 2, 13, or (2 × 13)F_1 PEC (0.1 × 10^6). The incorporation of [^{3}H]-TdR was determined on day 4 of the second culture.

[b] Cpm/Well; each value is the mean of three determinations.

DISCUSSION

Although the reactivity of T lymphocytes with autologous or syngeneic non-T cells has been extensively studied in both human and murine models for several years, the biological significance of this *in vitro* phenomenon and the nature of the target antigens are still unclear (1–11). The reactivity of guinea pig T cells with syngeneic non-T cells resembles in many respects what has been described in the human autologous MLR. A peak proliferative response was seen at 8 days of primary culture and an enhanced secondary response was seen on day 4. The syngeneic MLR also exhibited specificity in that T cells cultured with syngeneic PEC responded well to syngeneic PEC in the secondary culture, but displayed an absent or diminished response to allogeneic stimulation. A similar situation has been described in the autologous MLR in man (7). The magnitude of the proliferative response seen in the secondary syngeneic MLR approached in many experiments the magnitude of the response in the secondary allogeneic MLR. It should also be noted that, as in the studies of the human autologous MLR (6), high ratios of stimulator to responder cells were required to generate the guinea pig syngeneic MLR (unpublished observations). In our previous studies (13) in which we used lower ratios of stimulator to responder to prime for antigen-specific T cell proliferative responses *in vitro* very little reactivity was seen to PEC in the absence of antigen.

The responding cell population in the guinea pig syngeneic MLR was of T cell origin since extensively column passed lymph node cell preparations that contained less than 2% immunoglobulin-bearing cells gave vigorous proliferative responses. Furthermore, enriched populations of guinea pig B cells obtained by depleting T cells by rosette formation with rabbit erythrocytes (23) failed to respond (results not shown). The stimulator cell in the guinea pig system was present in unpurified PEC populations and in adherent populations prepared from PEC or spleen. Both unseparated spleen and lymph node cells failed to stimulate in spite of the fact that both these populations contain 30 to 50% B cells. We have tentatively concluded that the stimulator cell in the guinea pig syngeneic MLR is not a typical B lymphocyte. It should be noted, however, that we did not use purified B lymphocytes prepared from spleen or lymph nodes as stimulators. We have thus far been

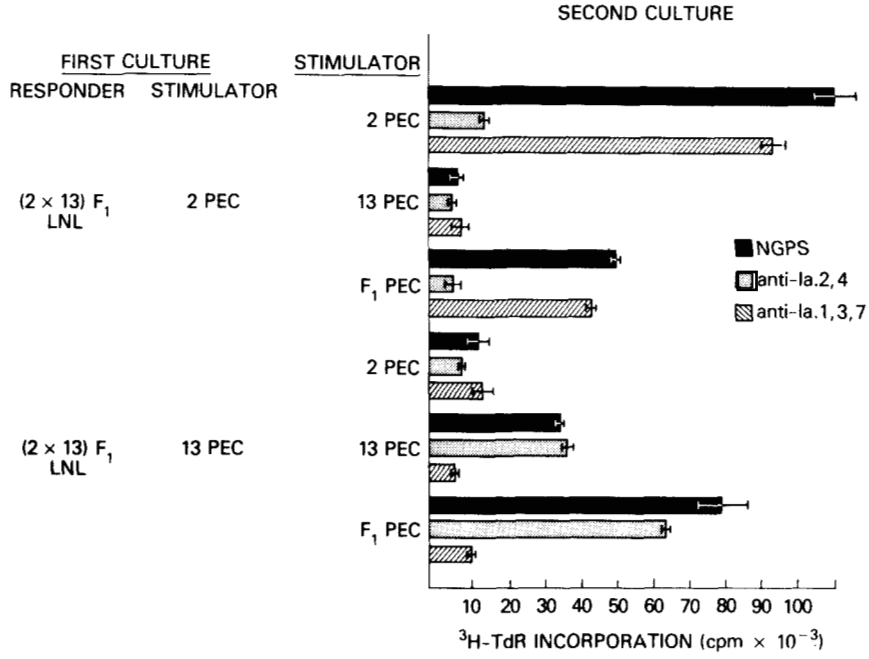

Figure 2. Inhibition of the syngeneic MLR with anti-Ia serum. (2 × 13)F_1 LNL (5 × 10^6) were cultured with parental PEC (2.5 × 10^6) for 14 days in the presence of 5% NGPS. Cells recovered from these cultures (0.1 × 10^6) were then restimulated with strain 2, 13, or (2 × 13)F_1 PEC (0.1 × 10^6) in the presence of 5% NGPS or anti-Ia serum. The incorporation of [^{3}H]-TdR was determined on day 4 of the second culture and is expressed as cpm × 10^{-3}/well; each value is the mean of three determinations.

unsuccessful in the preparation of B cell populations that are free of contaminating macrophages. It is unlikely that the failure of unseparated spleen or lymph node cells to stimulate purified T cells was secondary to the presence of suppressor cells in those populations since mixtures of spleen or lymph node cells with PEC stimulated a normal syngeneic MLR (results not shown).

Considerable controversy exists on the nature of the stimulator cell of the human autologous MLR. Some investigators have claimed that most of the activity is in the L cell or K cell population (6), the immunoglobulin-positive population (24), or in the monocyte and B cell but not the L cell population (17). Since the population of adherent PEC used in our studies are composed of greater than 95% macrophages by morphologic and phagocytic criteria, it is likely that the stimulator cell belongs to the monocyte-macrophage lineage. However, it should be noted that the stimulator cell belonged to the subpopulation (~10- to 20%) of guinea pig macrophages that bear Ia antigens. The cells responsible for the presentation of soluble protein antigens to immune T cells and for presentation of alloantigen in the allogeneic MLR are also present in this "Ia-positive" subpopulation of adherent PEC (19). It is not yet clear whether the presence of Ia antigens reflects a stage in monocyte-macrophage differentiation or whether Ia-bearing cells are of a different cell lineage. In this regard, Steinman and Witmer (25) have recently demonstrated that dendritic cells isolated from mouse spleen bear Ia antigens and are potent stimulators of both the allogeneic and syngeneic MLR (Steinman, R., personal communication).

Preliminary typing studies in man have suggested that the products of the HLA-D locus are important in the autologous MLR (8, 9). Although the failure of the Ia-negative PEC to stimulate the syngeneic MLR suggested that products of the guinea pig I-region were the target antigens in this reaction, more direct proof of the importance of Ia antigens was obtained from studies of the reactivity of F_1 T cells with parental PEC. F_1 T cells positively selected with PEC from one parent demonstrated an enhanced proliferative response to PEC of that parent in the second culture. When these haplotype restricted F_1 T cells were challenged with F_1 PEC, the response was specifically inhibited by anti-Ia sera directed to the Ia antigens of the strain of PEC used in the first culture. It is likely that the anti-Ia sera were acting on the stimulator macrophage because pretreatment of the responding T cells with anti-Ia serum in the presence of C had no effect or an enhancing effect on their subsequent response in the syngeneic MLR.

Studies in two immunologic diseases suggest that the reactive cell in the syngeneic MLR plays an important role in immunoregulation *in vivo*. Sakane *et al.* (17) have shown that the autologous MLR was impaired in patients with active systemic lupus erythematosus and Smith and Pasternak (26) have shown that the syngeneic MLR is absent in NZB mice. It is unlikely that the impaired response in these two studies was secondary to generalized immunosuppressive state since a normal allogeneic MLR was observed. Conflicting results have been obtained from other studies of the functional role of the human T cell reactive in the autologous MLR. The reactive cell has been deleted from proliferating populations by suicide with bromodeoxyuridine (BUdR) and light treatment and two groups have shown that the remaining cells respond normally in the allogeneic MLR (8, 10). However, Hausman and Stobo (8) have shown that the depleted cells were deficient in T-helper cell activity, while Sakane and Green (10) demonstrated a depletion of T suppressor function. Smith and Knowlton have also shown that T cells activated in the autologous MLR may exhibit suppressor activity (11).

Our observations that the characteristics of the stimulator cell and the genetic requirements for the activation of the syngeneic MLR precisely resemble the requirements for antigen-specific T cell activation have suggested a number of different possibilities for the functional role of the reactive cell in the syngeneic MLR. First, one must consider that the proliferative response observed *in vitro* merely represents a secondary response to a foreign antigen carried into the culture from the animal. However, the kinetics of the reaction are clearly different from the typical *in vitro* secondary response in the guinea pig that peaks on day 3 or 4 of culture. It is much more difficult to rule out the possibility of a primary response to a virus or a degradation product of normal serum. In order to avoid exposure to foreign serum proteins we have used NGPS instead of fetal calf serum not only for the culture but also for column purification of T cells. A second possibility is that the syngeneic MLR represents primary sensitization to a non-T cell or macrophage-specific antigen to which tolerance is maintained *in vivo* but not *in vitro*. An antigen of this type need not be polymorphic since the genetic restriction on the response of the F_1 T cell would be generated because the antigen would be recognized in association with self Ia. We have previously documented T cell sensitization against antibody modified B.1 antigen of the guinea pig MHC (the homologue of the mouse H-2K or H-2D antigens expressed by both strain 2 and strain 13 guinea pigs), which appeared to be regulated by I-region products (27). Thus, $(2 \times 13)F_1$ T cells sensitized with anti-B.1-treated parental macrophages could be restimulated only with anti-B.1-treated macrophages of the parental type used for initial sensitization, but not with those of the other parent.

A third possible explanation for the syngeneic MLR is derived from the studies of Zinkernagel *et al.* (28), which postulate a dual recognition model for the T cell receptor. According to this model, T cells differentiate recognition structures for self in the thymus that are specific for the histocompatibility antigens expressed by the thymic epithelium. The recognition of self H-2 differentiates independently of the recognition of any foreign antigen. If T cells do indeed express a receptor for self, it is possible that the syngeneic MLR represents T cell activation *in vitro* mediated through this low affinity anti-self receptor. This hypothesis is subject to experimental testing since one might predict that elimination of the clones of F_1 T cells reactive with the Ia antigens of one parent (by BUdR and light treatment) in the syngeneic MLR would lead to elimination of the clones capable of subsequently being sensitized to all foreign antigens in association with the Ia antigens of that parent, but not the other parent. Experiments of this type are now in progress.

Acknowledgments. We thank Ms. C. Chan for expert technical assistance and Dr. I. Green for helpful discussions and careful review of the manuscript.

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