

# MONOCLONAL ANTIBODIES TO GUINEA PIG Ia ANTIGENS

## II. Effect on Alloantigen-, Antigen-, and Mitogen-induced T Lymphocyte Proliferation In Vitro

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The major histocompatibility complex (MHC)<sup>1</sup>-linked immune response (*Ir*) genes control the capacity of an animal to mount a T cell-dependent immune response and are closely linked to genes that code for a group of cell surface antigens termed *I* region-associated (Ia) antigens (1). Alloantibodies to Ia antigens have proven to be extremely useful tools in defining the relationship between Ia antigens and *Ir* gene product function (2). Studies of the effects of anti-Ia antibody on T cell-proliferative responses in vitro have suggested that the Ia antigens themselves are the products of the *Ir* genes. Thus, it was initially demonstrated in the guinea pig (3) and later in the mouse (4) that the capacity of F<sub>1</sub> macrophages to present antigens to (nonresponder × responder)F<sub>1</sub> T cells can be blocked by anti-Ia antibodies specific for antigens of the responder parent, but not by anti-Ia antibodies specific for antigens of the nonresponder parent.

Antibodies raised by cross-immunization of different strains within a species are restricted in their specificity to polymorphic determinants varying between the individual strains. Nonpolymorphic regions on Ia antigens can be recognized by xenoantisera. The recently introduced hybridoma technique of Köhler and Milstein (5) provides a tool for the production of monoclonal xenogeneic antibodies and allows one to dissect the immune response to a complex antigen into its individual components. We have prepared a number of monoclonal antibodies to guinea pig Ia antigens.<sup>2</sup> Hybrids were obtained after immunization of BALB/c mice with Ia-positive EN-L2C leukemia cells of strain 2 guinea pigs and fusion of spleen cells to the NS-1 myeloma line. The secreted antibodies detected either alloantigenic determinants of strain 2 Ia antigens or common determinants present on both guinea pig strain 2 and strain 13 Ia antigens. In this report, the individual monoclonal antibodies were tested

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<sup>1</sup> Abbreviations used in this paper: (2 × 13)F<sub>1</sub>, (strain 2 × strain 13)F<sub>1</sub>; CFA, complete Freund's adjuvant; Δcpm, counts per minute with antigen-pulsed peritoneal exudate cells – counts per minute with unpulsed peritoneal exudate cells; DNP, 2,4-dinitrophenyl; FCS, fetal calf serum; GL, a copolymer of L-glutamic acid (60%) and L-lysine (40%); GT, a copolymer of L-glutamic acid (50%) and L-tyrosine (50%); [<sup>3</sup>H]TdR, tritiated thymidine; LNL, enriched column-purified T lymphocytes from lymph nodes; MHC, major histocompatibility complex; MLR, mixed leukocyte reaction(s); NIH, National Institutes of Health; NGPS, normal guinea pig serum; OVA, ovalbumin; PEC, peritoneal exudate cells; PEL, peritoneal exudate lymphocytes; PHA, phytohemagglutinin; PPD, purified protein derivative of tuberculin.

<sup>2</sup> Burger, R. B., L. Clement, J. Schroer, J. Chiba, and E. M. Shevach. Monoclonal antibodies to guinea pig Ia antigens. I. Production, serological, and immunochemical characterization. Manuscript submitted for publication.

for their inhibitory effects on antigen-, alloantigen-, and mitogen-induced T cell proliferation. The data demonstrate that monoclonal anti-Ia antibodies are as capable of inhibiting T-cell activation by these stimuli as alloantibodies to Ia antigens raised by cross-immunization of inbred strain 2 and strain 13 animals. In addition, a detailed analysis of the effects of a single monoclonal antibody on the responses to several antigens demonstrated a selective pattern of inhibition in that the responses to some, but not all, antigens were inhibited. These results suggest that monoclonal anti-Ia antibodies may be able to discriminate between functionally different regions on individual Ia molecules involved in antigen presentation.

### Materials and Methods

**Animals.** Inbred strain 2, strain 13, and (strain 2  $\times$  strain 13) $F_1$  [(2  $\times$  13) $F_1$ ] guinea pigs were obtained from the Division of Research Services, National Institutes of Health (NIH), Bethesda, Md.

**Monoclonal Antibodies to Guinea Pig Ia Antigens.** The production of monoclonal antibodies directed to guinea pig Ia antigens and their properties is described in detail elsewhere.<sup>2</sup> A hybrid myeloma line provided by Ms. R. Lieberman, NIH, which secreted an IgM antibody directed to inulin in addition to the parental ( $\gamma_1$ ,  $\kappa$ ) antibody of the P3X63 Ag8 myeloma line, was used for the production of a control ascites. In all experiments, this control ascitic fluid was used in parallel to exclude any nonspecific effects.

**Alloantibodies to Ia Antigens.** 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 described previously (6).

**Antigens.** A copolymer of L-glutamic acid (60%) and L-lysine (40%) (GL), with an average 40,000 mol wt, was purchased from New England Nuclear, Boston, Mass. Ovalbumin (OVA) and a copolymer of L-glutamic acid (50%) and L-tyrosine (50%) (GT), 14,500 mol wt, were purchased from Miles Laboratories, Inc., Miles Research Division, Kankakee, Ill. Purified protein derivative of tuberculin (PPD) was purchased from Connaught Medical Research Laboratory, Willowdale, Ontario, Canada. 2,4-dinitrophenyl (DNP)-GL was prepared as described by Janeway and Paul (7).

**Immunizations.** Solutions of each antigen in saline were emulsified with an equal volume of complete Freund's adjuvant (CFA) that contained 0.4 mg/ml *Mycobacterium tuberculosis* H37Ra (Difco Laboratories, Detroit, Mich.). Each animal received 0.1 ml of emulsion in each footpad. Strain 2 animals received a total of 100  $\mu$ g of DNP-GL and 100  $\mu$ g of OVA; strain 13 animals received 100  $\mu$ g of OVA and 500  $\mu$ g of GT.

**Preparation of Peritoneal Exudate Cells and T Lymphocytes.** 2–4 wk after immunization, the animals were injected intraperitoneally with 25 ml of sterile mineral oil (Marcol 52; Humble Oil & Refining Co., Houston, Tex.). 4 d later, the resulting peritoneal exudate was harvested. This cell population consisted of ~75% macrophages, 10% granulocytes, and 15% lymphocytes. Peritoneal exudate lymphocytes (PEL) were obtained by passing the peritoneal exudate cells (PEC) over a rayon-wool-adherence column (8). The resulting cell population was composed of 80–90% T lymphocytes, 10–20% macrophages, and <5% B lymphocytes. Enriched column-purified T lymphocytes from lymph nodes (LNL) were obtained by passing cells of the regional lymph nodes of immunized guinea pigs over a rayon-wool column (8). This cell population consisted of 85–95% T lymphocytes and 10–15% B lymphocytes.

**Proliferative Response of Immune T Lymphocytes to Soluble Protein Antigens or to Mitogen.** Antigen- or mitogen-induced proliferation of antigen-primed T cells was measured by incubation with antigen- or mitogen-pulsed macrophages. RPMI-1640 medium (Grand Island Biological Co., Grand Island, N. Y.) that contained L-glutamine (300  $\mu$ g/ml), penicillin (100 U/ml), streptomycin (100  $\mu$ g/ml), 5-fluorocytosine (1  $\mu$ g/ml), 2-mercaptoethanol ( $5 \times 10^{-6}$  M), and 10% heat-inactivated fetal calf serum (FCS) was used. For antigen or mitogen pulsing, unfractionated mineral oil-induced PEC ( $10 \times 10^6$ /ml) from nonimmune guinea pigs were incubated in RPMI-1640 medium at 37°C for 1 h in the presence of 25  $\mu$ g/ml mitomycin C (Sigma Chemical Co., St. Louis, Mo.) and 100  $\mu$ g OVA, DNP-GL, GT, PPD, or phytohemagglutinin (PHA) (Burroughs Wellcome, Beckenham, Kent, England). The cells were then washed four times to

remove unbound antigen or mitogen. These antigen- or mitogen-pulsed PEC ( $1 \times 10^5$ ) were incubated in 0.2 ml of medium with PEL ( $1 \times 10^5$ - $2 \times 10^6$ ) from an immunized animal for 4 d in round-bottomed microtiter plates (Linbro Chemical Company, New Haven, Conn.).  $1.0 \mu\text{Ci}$  of tritiated thymidine ( $[^3\text{H}]\text{TdR}$ ) ( $6.7 \text{ Ci/mM}$  sp act; New England Nuclear) was added to each well 18 h before harvesting, and the amount of radioactivity incorporated into cellular DNA was determined with the aid of a semiautomated microharvesting device Mash II, Microbiological Associates, Walkersville, Md. The results of triplicate cultures are expressed as total counts per minute/culture or as counts per minute with antigen-pulsed PEC - counts per minute with unpulsed PEC ( $\Delta\text{cpm}$ ). The SEM of triplicate samples was never  $>10\%$  of the mean, and for simplicity, only the mean of triplicate samples is reported in the various proliferation assays.

*Mixed Leukocyte Reaction (MLR).* Mitomycin-treated PEC ( $1 \times 10^5$ ) were cultured for 6 d with rayon-wool-purified LNL ( $3 \times 10^5$ ) in flat-bottomed microtiter plates in 0.2 ml of medium that contained 5% heat-inactivated normal guinea pig serum (NGPS) or FCS.  $[^3\text{H}]\text{TdR}$  incorporation was measured as described above.

## Results

*Reactivity of the Monoclonal Antibodies with the I Subregions of Strain 2 and Strain 13 Guinea Pigs.* To facilitate the interpretation of the studies to be presented on the effects of the monoclonal antibodies on T lymphocyte function, we will first summarize the results of our serologic and biochemical studies<sup>2</sup> on the reactivity of the monoclonal anti-Ia antibodies with the different I subregions of strain 2 and strain 13 guinea pigs. The I region of the MHC of strain 2 animals has been divided into two or possibly three subregions (9). The Ia.2 antigen was present on a molecule in which the 33,000- and 25,000-dalton chains were noncovalently associated, whereas the Ia.4,5 antigens were found on a distinct molecule in which the 33,000- and 25,000-dalton chains were linked by disulfide bonds; the subregion assignment of Ia.6 has not been made; and the existence of a third subregion in strain 2 animals was inferred from the data obtained from strain 13 guinea pigs. All four monoclonals were cytolytic for strain 2 cells at dilutions as high as 1/10,000. Monoclonal antibody 22C4 was shown in immunoprecipitation studies to react with the Ia.2-bearing molecule as well as the molecule that bore Ia.4,5; in contrast, 27E7 was shown in sequential precipitation studies to react only with the Ia.2-bearing and not the Ia.4,5-bearing molecule. Monoclonals 25E3 and 25E11 were shown in a radioactive binding study with  $^{125}\text{I}$ -labeled  $\text{F}(\text{ab}')_2$  rabbit anti-mouse Ig to react with alloantigenic determinants of strain 2 Ia antigens in that they failed to react with strain 13 cells and that their binding to strain 2 cells could be inhibited by 13 anti-2 serum (anti-Ia.2,4); however, as they were inactive in immunoprecipitation studies, we could not specifically determine against which I-subregion of the strain 2 I-region they were reactive.

The strain 13 I region can be divided into three subregions: the Ia.3,5 antigens were found on a molecule in which the 33,000- and 25,000-dalton chains were not covalently associated, whereas Ia.7 was present on a molecule in which the two chains were covalently linked, and Ia.1,6 was found on a single chain molecule of 26,000 daltons. Of the two monoclonals reactive with strain 13 cells (22C4 and 27E7), only one, 22C4, was cytolytic in the presence of complement. However, both 27E7 and 22C4 could be shown to react with the products of all three subregions because preprecipitation of  $^3\text{H}$ -labeled extracts of strain 13 cells with either of these monoclonals removed all reactivity for 2 anti-13 serum (anti-Ia.1,3,7).

*Inhibition of the MLR by Monoclonal Anti-Ia Antibodies.* Alloantisera to guinea pig Ia

antigens inhibit T lymphocyte proliferation induced by allogeneic macrophages (10). The four monoclonal antibodies (22C4, 25E3, 25E11, and 27E7) to guinea pig Ia antigens were compared with alloantibodies for inhibition of the MLR. LNL of one strain were cultured for 6 d with mitomycin C-treated syngeneic or allogeneic macrophages. Ascitic fluids produced from the individual hybrid cell lines or the control ascites were diluted in heat-inactivated NGPS and added to the cultures to give a final serum concentration of 5%. The antibody dilution given in the Tables or Figures represents the final dilution of the ascitic fluids in the culture. [<sup>3</sup>H]TdR incorporation was determined in parallel cultures in the presence of NGPS alone, 13 anti-2, or 2 anti-13 alloantisera.

The results of a representative experiment are shown in Table I. All four monoclonal anti-Ia antibodies inhibited the MLR when strain 2 macrophages were used as stimulator cells and strain 13 LNL as responder cells. In contrast, only antibodies 22C4 and 27E7 inhibited the MLR between strain 2 LNL as responder and strain 13 macrophages as stimulator cells. The control ascitic fluid in a number of proliferation experiments never exhibited any significant inhibitory effects. The experiment in Table I was performed at a 1/100 final dilution of the four monoclonal antibodies. In several experiments of this type performed at this dilution of ascites, [<sup>3</sup>H]TdR incorporation was inhibited by 50–70%. However, the inhibitory effect of the monoclonal antibodies on the MLR was rapidly lost upon further dilution and was no longer present at a dilution of 1/800. Mixing of the monoclonal antibodies in different combinations while maintaining the same final antibody concentration in the cultures did not result in any additional inhibitory effects. The observed inhibitory pattern in the MLR parallels the reactivity of the corresponding antibodies in binding or immunoprecipitation experiments. All four antibodies were shown to react with strain 2 Ia antigens, and all inhibited the MLR using strain 2 macrophages as stimulators. On the other hand, only 22C4 and 27E7, which proved to recognize common determinants present on both strain 13 and strain 2 Ia antigens, also inhibited the strain 2 LNL proliferative response to strain 13 macrophages. The unidirectional inhibition of the MLR by 25E3 and 25E11, which reacted with alloantigenic

TABLE I  
*Inhibition of the MLR by Monoclonal Anti-Ia Antibody*

	13* > 2‡	2 > 13	13 > 13	2 > 2
			<i>cpm</i>	
22C4	<i>43,042</i>	<i>37,981</i>	1,953	768
25E3	<i>47,942</i>	<i>68,765</i>	1,906	2,030
25E11	<i>51,838</i>	<i>64,425</i>	788	2,113
27E7	<i>48,288</i>	<i>22,713</i>	621	789
Control ascites	98,076	64,759	1,402	1,264
NGPS	105,423	71,840	953	1,424
2 anti-13	106,423	<i>8,000</i>	835	1,437
13 anti-2	30,961	65,000	994	1,291

LNL ( $3 \times 10^5$ ) were cultured with syngeneic or allogeneic PEC ( $1 \times 10^5$ ) for 5 d in the presence of a 1/100 dilution of ascites or alloantibody. Results are expressed as counts per minute [<sup>3</sup>H]TdR incorporation. Results in italics represent significant inhibition.

\* Responder.

‡ Stimulator.

determinants of strain 2 Ia molecules, confirms with monoclonal reagents that anti-Ia antibodies specific for the stimulator cells and not for the responder cells inhibit alloantigen-induced T cell proliferation.

*The Effect of the Monoclonal Anti-Ia Antibodies on the Antigen- and Mitogen-induced Proliferative Response of Strain 2 PEL.* The proliferative response of PEL to stimulation by a large number of antigens in vitro is markedly depressed in the presence of alloantisera directed to Ia antigens (11). The xenogeneic monoclonal anti-Ia antibodies were similarly tested for their inhibitory activity on T lymphocyte proliferation. As the antibody-secreting hybrid cell lines were produced after immunization with strain 2 cells, they were first tested for their effect on antigen- or mitogen-induced proliferation of strain 2 PEL in vitro. PEL from immune strain 2 guinea pigs were cultured with antigen-pulsed macrophages for 3 d in the presence of 10% FCS that contained the monoclonal anti-Ia antibodies or the control ascites at different dilutions. A typical concentration-dependent inhibition of the PPD-induced proliferative response by monoclonal antibodies 22C4 and 27E7 is shown in Fig. 1. Significant inhibition was seen at dilutions of 1/2,500. In contrast, antibodies 25E3 and 25E11 had little, if any, effect on the PPD-induced proliferative response of strain 2 PEL. The proliferative response in the presence of the control ascites even at a high concentration (1/30 final dilution in culture) was in the range of the control culture that contained FCS alone. The background proliferation in the absence of PPD was approximately the same in FCS or in ascites-containing cultures.

The effect of the monoclonal anti-Ia antibodies on the proliferative response of strain 2 PEL to the antigens OVA and GL and the mitogen PHA was then tested. GL

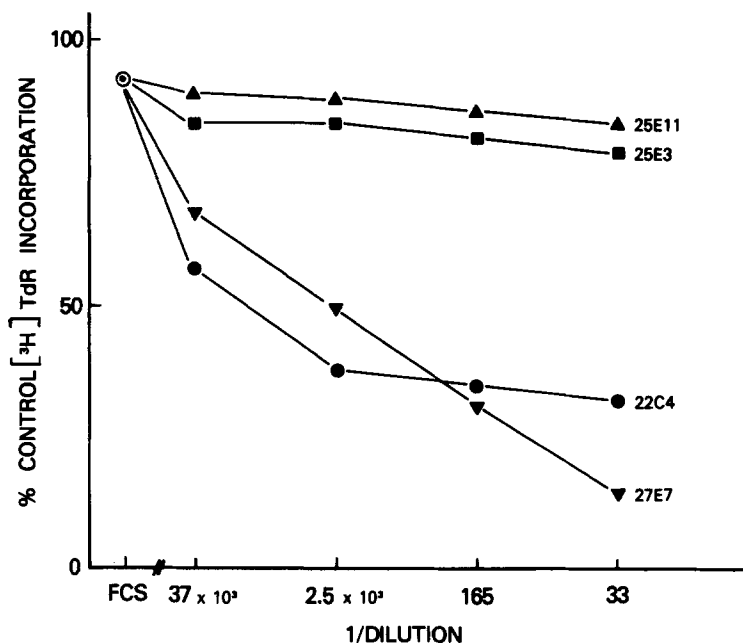


FIG. 1. Dose-dependent inhibition of the proliferative response of strain 2 PEL to PPD-pulsed macrophages by monoclonal anti-Ia antibody. Strain 2 PEL ( $2 \times 10^6$ ) were cultured with syngeneic PEC ( $1 \times 10^6$ ) in the presence of different dilutions of the anti-Ia antibodies. Results are expressed as  $\Delta$ cpm [ $^3$ H]TdR incorporation.

is an antigen, the response to which is controlled by an *Ir* gene linked to the Ia.2 antigen of the strain 2 MHC (9), whereas the responses to OVA, PPD, or PHA are not under unigenic *Ir* gene control. In a few experiments, DNP-GL was used instead of nonsubstituted GL and gave the same results as GL. Table II represents a typical result obtained in a large number of experiments. All cultures contained 10% FCS and a final concentration of 1/800 of each monoclonal antibody. The effects of a 1/100 dilution of 13 anti-2 serum on these responses were studied in parallel cultures. The proliferative response to GL-pulsed macrophages was significantly inhibited by 25E3, 25E11, and 27E7 as well as by 13 anti-2 serum. Surprisingly, monoclonal 22C4, which, as our previous biochemical studies demonstrated, reacted specifically with the Ia.2-bearing molecule, failed to inhibit the proliferative response to GL to a significant extent. The response to OVA was only slightly (25%) inhibited by all the monoclonals tested. Again, as demonstrated in Fig. 1, monoclonals 22C4 and 27E7, but not 25E3 and 25E11, produced a 60–70% inhibition of the response to PPD. Only 27E7 produced a significant inhibition of the proliferative response to PHA-pulsed macrophages.

*The Effect of the Monoclonal Anti-Ia Antibodies on the Antigen- and Mitogen-induced Proliferative Responses of Strain 13 PEL.* Although all the monoclonals were raised against strain 2 cells, antibodies 22C4 and 27E7 were shown to recognize common or framework determinants of Ia antigens present on all strain 13 Ia molecules. We therefore tested the effects of these antibodies, as well as 25E3, 25E11, and 2 anti-13 serum on the proliferative response of strain 13 PEL to macrophages pulsed with the protein antigens GT, OVA, and PPD, and the mitogen PHA (Table III). As in the studies with strain 2 PEL, all cultures were performed in 10% FCS that contained each monoclonal antibody in a final dilution of 1/800. The proliferative response to GT was significantly inhibited by monoclonals 22C4 and 27E7, and both 22C4 and 27E7 produced a 50–60% inhibition of the response to PPD-pulsed macrophages. As expected, monoclonals 25E3 and 25E11, which in both binding and cytotoxicity assays were shown to be specific for alloantigenic determinants of strain 2 Ia molecules, failed to produce any inhibition of the responses of strain 13 PEL.

*The Effect of the Monoclonal Anti-Ia Antibodies on the Antigen- and Mitogen-induced*

TABLE II  
*Effect of the Monoclonal Anti-Ia Antibodies on the Proliferative Response of Strain 2 PEL*

	Stimulus			
	GL	OVA	PPD	PHA
			<i>Δcpm</i>	
22C4	28,464	44,144	35,217	112,432
25E3	<i>9,666</i>	46,907	89,954	89,957
25E11	<i>9,691</i>	45,059	85,678	98,303
27E7	<i>7,123</i>	38,763	<i>31,219</i>	<i>49,632</i>
Control ascites	33,740	58,085	101,853	85,897
FCS	31,050	61,464	91,767	103,939
13 anti-2	<i>5,697</i>	<i>30,635</i>	<i>49,654</i>	<i>62,170</i>

PEL ( $2 \times 10^5$ ) were cultured for 3 d with antigen- or mitogen-pulsed PEC ( $1 \times 10^5$ ) in the presence of a 1/100 dilution of alloantibody. Results are expressed as  $\Delta\text{cpm}$  [ $^3\text{H}$ ]TdR incorporation. Results in italics represent significant inhibition.

TABLE III  
*Effect of Monoclonal Anti-Ia Antibodies on the Proliferative Response of Strain 13 PEL*

	Stimulus			
	GT	OVA	PPD	PHA
	<i>Δcpm</i>			
22C4	25,167	80,010	59,429	171,998
25E3	72,805	86,953	105,363	175,409
25E11	65,308	81,735	121,875	198,002
27E7	33,942	32,491	45,555	139,775
Control ascites	67,786	90,973	132,109	209,339
FCS	68,992	85,634	121,468	225,333
2 anti-13	38,872	41,237	134,369	96,135

PEL ( $2 \times 10^5$ ) were cultured for 3 d with antigen- or mitogen-pulsed PEC ( $1 \times 10^5$ ) in the presence of a 1/800 dilution of alloantibody. Results are expressed as  $\Delta$ cpm [ $^3$ H]TdR incorporation. Results in italics represent significant inhibition.

*Proliferative Responses of (2 × 13)F<sub>1</sub> PEL.* Anti-Ia sera raised by cross-immunization of strain 2 and strain 13 animals were shown to inhibit specifically the activation of T lymphocytes from immune (2 × 13)F<sub>1</sub> guinea pigs, by antigens the response to which is controlled by *I*r genes (2). It was therefore of interest to repeat these studies with the monoclonal anti-Ia antibodies. In Fig. 2, we have presented a concentration curve of the effects of two monoclonals, 22C4 and 25E11, on the proliferative responses of (2 × 13)F<sub>1</sub> T cells to GL and GT. Monoclonal antibody 22C4 produced a marked inhibition of the proliferative response to GT as well as to PPD, but had no effect on the proliferative response to GL- or to PHA-pulsed macrophages. In contrast, monoclonal 25E11 produced a selective inhibition of the T cell response to GL and had no effect on the response to the other antigens or the mitogen PHA.

In Table IV, we have presented the results of studies performed with all four monoclonal antibodies at a final concentration of 1/800. As seen in studies with inbred strain 13 PEL (Table III), only monoclonals 22C4 and 27E7 produced a marked inhibition of the response to GT-pulsed macrophages; and as seen in the studies with strain 2 PEL (Table II), only 22C4 failed to inhibit the proliferative response to GL-pulsed macrophages. The proliferative response to PPD, which was inhibited in both inbred strains by 22C4 and 27E7, was also inhibited by these monoclonals when F<sub>1</sub> cells were tested. Stimulation by PHA was, as in strain 2 and strain 13, only inhibited by 27E7. Cultivation in the presence of mixtures of the individual antibodies in several combinations did not lead to any substantial additive inhibitory effect.

### Discussion

One useful approach to the further analysis of the function of the Ia antigens in immunocompetent cell interactions and, in particular, to the dissection of the relationship between Ia antigens and *I*r gene products has been to inhibit various in vitro assays of T lymphocyte function with anti-Ia antibodies. Our early studies in the guinea pig that demonstrated that anti-Ia antibodies specifically inhibited the activation of T lymphocytes from immune guinea pigs by antigens, the response to which is controlled by *I*r genes, strongly suggested that the Ia antigens themselves were

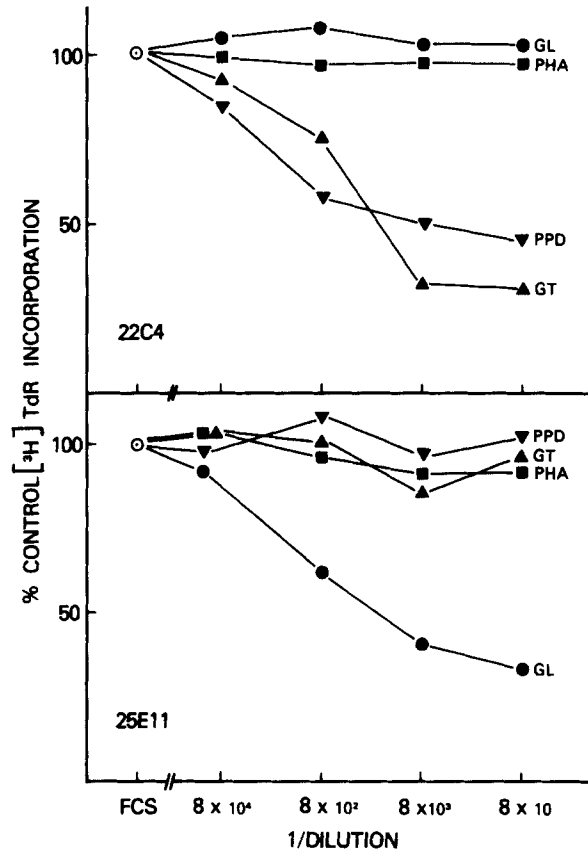


FIG. 2. Inhibition of the proliferative response of  $(2 \times 13)F_1$  PEL to antigen- or mitogen-pulsed PEC.  $F_1$  PEL ( $2 \times 10^5$ ) were cultured with antigen- or mitogen-pulsed  $F_1$  PEC ( $1 \times 10^5$ ) in the presence of the indicated dilution of 22C4, 25E11, or the control ascites. Results are expressed as the percentage of control  $[^3H]TdR$  incorporation

$$= 100 \times \frac{\Delta cpm \text{ in anti-Ia ascites}}{\Delta cpm \text{ in control ascites}}$$

TABLE IV

Effect of Monoclonal Anti-Ia Antibodies on the Proliferative Response of  $(2 \times 13)F_1$  PEL

	Stimulus			
	GL	GT	PPD	PHA
	$\Delta cpm$			
22C4	39,300	5,749	44,031	62,440
25E3	14,011	12,399	82,398	54,577
25E11	10,834	13,867	109,836	57,321
27E7	5,063	6,908	48,438	29,867
Control ascites	32,507	15,058	95,237	65,438

$(2 \times 13)F_1$  PEL ( $2 \times 10^5$ ) were cultured for 3 d with antigen- or mitogen-pulsed syngeneic PEC ( $1 \times 10^5$ ) in the presence of a 1/800 dilution of the anti-Ia or the control ascites. Results are expressed as  $\Delta cpm$   $[^3H]TdR$  incorporation. Results in italics represent significant inhibition.



identical to the products of the *Ir* genes (2). In general, studies of the effects of anti-Ia antibodies on lymphocyte function in vitro have been performed with alloantibodies that represent a mixture of different antibodies of several subclasses with different biologic properties directed to an unknown number of antigenic determinants on a given Ia antigen molecule. It is even possible that some of the available antisera that are defined as monospecific on a genetic basis might contain antibodies to as yet undefined products of the *I* region, and that the biological effects of these sera may be mediated by interaction with these unknown antigens rather than with what has been defined as an Ia antigen by biochemical criteria. The use of monoclonal anti-Ia antibodies, which exhibit strict specificity and which do not vary in isotype, affinity, or other biologic properties, affords an opportunity to more precisely analyze the effects of anti-Ia antibodies on T lymphocyte activation in vitro and to directly address some of the objections raised to the use of alloantibodies.

A number of investigators have already used monoclonal anti-MHC antibodies to inhibit T cell responses in vitro. Thus, monoclonal antibodies to species-specific nonpolymorphic determinants on the HLA-A, -B, and -C chains have been shown to inhibit the specific cytotoxic response of human T cells that were sensitized to influenza virus-infected cells (12). In contrast, monoclonal antibodies to polymorphic HLA-B determinants failed to inhibit the cytotoxic activity. In the mouse, monoclonal antibody reacting with public specificities of *H-2K<sup>k</sup>* was shown to inhibit T cell-mediated allogeneic cytotoxicity directed against products of the *H-2K<sup>k</sup>* region and to inhibit the *H-2K<sup>k</sup>*-restricted cytotoxicity of minor histocompatibility antigens (13). All of these studies have examined the effects of monoclonal antibodies on cytotoxic T cell function directed to the products of the *H-2K* and *D* genes and their analogues in other species. Little data are available at present on the effects of monoclonal anti-Ia antibodies on T lymphocyte function.

We have demonstrated, with the aid of a highly selective screening system, that xenogeneic monoclonal antibodies to guinea pig Ia antigens could readily be produced. A detailed serologic analysis demonstrated that the monoclonal antibodies recognized either framework determinants shared by both strain 2 and strain 13 Ia antigens or alloantigenic determinants of strain 2 Ia antigens. In addition, an analysis of the reactivity pattern of the monoclonals with strain 2 Ia antigens suggested that a number of distinct determinants on strain 2 Ia antigens were identified by the different monoclonal reagents.<sup>2</sup> It was therefore of interest to compare these different monoclonal reagents with alloantibodies to Ia antigens with regard to their effects on T-lymphocyte proliferation in vitro.

In the guinea pig MLR (9), as well as in the mouse (14) and in man (15), it is likely that the Ia antigens themselves are the stimulatory antigens because alloantibodies to stimulator-, but not responder-, cell Ia that are present continuously in the cultures inhibit the MLR, and because pretreatment of macrophages with anti-Ia antibody and complement results in the inability of those cells remaining alive to act as stimulator cells (16). In general, the results of our studies on the inhibition of the MLR with the monoclonal antibodies are consistent with this model. All four monoclonal antibodies tested reacted with strain 2 Ia antigens and all four were capable of inhibiting the strain 13 against strain 2 MLR by 50–70%. Similarly, in binding and cytotoxicity assays, only 22C4 and 27E7 were shown to be reactive with

strain 13 Ia antigens, and only these two monoclonals were capable of inhibiting the strain 2 against strain 13 MLR.

A number of points should be emphasized about the results of the MLR studies. First, monoclonal antibodies 25E3 and 25E11, which were shown to be directed against alloantigenic determinants of strain 2 Ia molecules, specifically inhibited the strain 13 against strain 2 MLR, thus confirming with monoclonal reagents that Ia antigen molecules on stimulator, but not responder, cells are the target antigens for inhibition of the MLR. Second, although it is difficult to directly compare the monoclonal reagents with alloantibodies, our general impression was that the degree of inhibition of the MLR produced by high concentrations of monoclonal reagents was less than that seen with high concentrations of alloantibody. Such a result might be anticipated in view of the likelihood that the alloantisera contain antibodies to multiple determinants on Ia antigen molecules, whereas the monoclonal reagents are much more restricted in specificity. However, it was surprising that combinations of the monoclonals failed to produce additive inhibitory effects, particularly in view of our results that 22C4, 27E7, and 25E3 recognize distinct antigenic determinants on strain 2 Ia molecules. It should also be noted that marked inhibition of antigen-induced proliferation was observed at dilutions of the monoclonals which failed to inhibit the MLR.

Before a detailed discussion of the effects of the monoclonals on antigen- and mitogen-induced T-cell activation can be presented, it is appropriate to review our previous studies on the effects of alloantibodies on antigen-induced T cell proliferative responses. We have previously shown that anti-Ia sera directed against multiple specificities of either the strain 2 or strain 13 *I* region produced a marked inhibition of the in vitro antigen-induced proliferative response, both to antigens, the response to which is under control of specific *I*r genes, and to antigens, the response to which is not under unigenic control (11); the proliferative response to PHA-pulsed macrophages was also inhibited by 50–60%. Anti-Ia sera directed to the products of a single *I* subregion were selective in their inhibitory profile and produced inhibition of the responses only to antigens linked to the *I* subregion against which the serum was directed (9). In general, antisera to the product of a single *I* subregion produced much less effect on the proliferative response to complex antigens, such as OVA and PPD, than that produced by 13 anti-2 serum or 2 anti-13 serum, which are reactive with all the subregions of the strain 2 or strain 13 *I* regions, respectively (9).

As all of the monoclonal antibodies tested behaved differently in their effects on antigen-induced proliferation; we will discuss each antibody or group of antibodies separately. Monoclonals 25E3 and 25E11 appeared to be similar to alloantibodies to the Ia.2 antigen of the strain 2 *I* region and specifically inhibited the response of strain 2 and (2 × 13)F<sub>1</sub> T cells to GL-pulsed macrophages. Although 25E3 and 25E11 were not active in immunoprecipitation studies and the molecular characteristics of their target antigens were not identified, the results of the functional studies clearly demonstrated that they react with Ia.2.

The results of the studies with monoclonals 27E7 and 22C4, which react with determinants present on both strain 2 and strain 13 *I* region products, were considerably more complex. 27E7 inhibited the proliferative response to most antigens tested and was the only monoclonal to significantly inhibit the proliferative response to the mitogen PHA. This monoclonal thus appears to mimic the effects of both 2 anti-13

serum and 13 anti-2 serum. It should be emphasized, however, that sequential precipitation studies demonstrated that 27E7 was reactive with all the products of the strain 13 *I* region, but was reactive only with the Ia.2- and not with the Ia.4,5-bearing molecule of the strain 2 *I* region.

The results of the studies with monoclonal 22C4 are of greatest interest. In immunoprecipitation studies, 22C4 appeared to recognize a framework or common determinant present on all Ia molecules of both strain 2 and strain 13 *I* regions. However, although 22C4 produced marked inhibition of the proliferative response of strain 2 T lymphocytes to PPD, it failed to produce any inhibition of the response to GL. Thus, although 22C4 reacts with the Ia.2-bearing molecule, it does not appear to recognize that part of the molecule that participates in the presentation of GL. Paradoxically, 22C4 was very effective in inhibiting the response of strain 13 or (2 × 13)F<sub>1</sub> lymphocytes to GT. Thus, because 22C4 was raised against GT nonresponder strain 2 cells, we might conclude that a portion of the Ia.1,6-bearing molecule involved in the presentation or recognition of GT on strain 13 cells must also be present on nonresponder strain 2 Ia molecules. Alternatively, we cannot exclude the possibility that, as has been shown in murine systems (17), two complementary Ia chains are necessary to mount a specific response to GT in strain 13, and that monoclonal 22C4 identifies a determinant present on one of these chains that is coded for in both strain 2 and strain 13 *I* regions.

It is likely, as has been reported with antigen-induced T cell proliferation (18), that the monoclonals exert their inhibitory effects by reacting with Ia molecules on the antigen-presenting macrophage. We cannot exclude the possibility that some of the inhibitory effects of the antibodies might be directed toward the T cell, because the primed guinea pig T cell responding in vitro in a secondary proliferation assay has been shown to bear Ia antigens with both alloantibodies (19) and monoclonal anti-Ia reagents (R. Burger and E. M. Shevach. Unpublished observations.). However, the specific and selective inhibition of T cell proliferation by the monoclonal antibodies argues strongly in favor of the macrophage as the cellular site of their action, because no evidence is available that argues for a participation of Ia antigens at the T cell surface in the specific recognition of antigen.

The results of our studies with the monoclonals are strongly in favor of the view that antibodies to the Ia antigens, rather than antibodies to unique determinants of *I**r* gene products, directly mediate the inhibition of T cell proliferation and that the Ia antigens themselves are the products of the *I**r* genes. Thus, in Fig. 2 we have clearly demonstrated with monoclonal antibodies that the proliferative response of (2 × 13)F<sub>1</sub> lymphocytes to antigen-pulsed F<sub>1</sub> macrophages can be inhibited by antibodies to responder, but not nonresponder, parental Ia antigens. This result is identical to that of our earlier studies performed with alloantisera (3).

A number of different mechanisms for the interaction of anti-Ia antibodies with Ia antigens on the macrophage surface should be considered. It is possible that anti-Ia antibodies lead to a generalized capping and shedding of Ia antigens and possibly associated determinants of the nominal antigen from the macrophage surface; alternatively, the anti-Ia antibodies may inhibit the mobility of Ia antigens in the cell membrane or lead to a loss of biological activity of the Ia molecule by altering the orientation of the Ia molecule in the membrane required for biological function. The results of the studies with monoclonal 27E7, which inhibited the proliferative response

to most antigens, the mitogen PHA, and alloantigens, are consistent with these explanations. On the other hand, the results of the studies with monoclonal 22C4, which inhibited the proliferative response to some, but not all, antigens, suggest a much more specific function of the Ia antigen molecule on the cell surface, that is, that the monoclonal antibody might react with different parts of the Ia molecules that have different functional roles. Certain parts of an Ia molecule might participate in the presentation of certain antigens or antigenic fragments but not others. These results are also consistent with the hypothesis proposed by Benacerraf (20) that states that specific *Ir* gene function dictates the ability of Ia molecules on macrophages to interact with antigens, and that Ia antigens on macrophages have a limited number of binding sites specific for unique amino acid sequences on antigens. Thus, 22C4 fails to react with that portion of the Ia.2 antigen molecule on the macrophage surface involved in the recognition or required for presentation of the copolymer GL. A larger panel of monoclonal antibodies should enable us to evaluate more fully this hypothesis and to correlate directly the presence of a given antigenic determinant on an Ia molecule with functional activity in antigen presentation and immunocompetent cell interaction.

### Summary

Four xenogenic monoclonal antibodies to guinea pig Ia antigens were tested for their inhibitory effects on antigen-, alloantigen-, and mitogen-induced T cell proliferation. All four monoclonal antibodies reacted with strain 2 Ia antigens, and all four were capable of inhibiting the strain 13 against strain 2 mixed leukocyte reaction (MLR) by 50–70%; the two monoclonals that reacted with strain 13 Ia antigens were also capable of inhibiting the strain 2 against strain 13 MLR. In contrast, an analysis of the effects of a single monoclonal antibody on the responses to several antigens demonstrated a selective pattern of inhibition in that the responses to some, but not all, antigens were inhibited. These results suggest that monoclonal antibodies react with different parts of Ia molecules that may have different functional roles and that certain parts of an Ia molecule participate in the presentation of certain antigens, whereas other regions of the same molecule present different antigens.

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