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The role of rabbit Ia molecules in immune functions as determined with the use of an anti-Ia monoclonal antibody

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Summary. We have produced a mouse anti-rabbit Ia monoclonal antibody (MAb) that detects an isotypic determinant on all rabbit Ia molecules. This MAb precipitates three polypeptide chains with molecular weights of 28,000, 31,000 and 35,000, corresponding to the Ia β, Ii and α chains, respectively. The anti-Ia MAb inhibits the mixed lymphocyte culture by 80%. In secondary *in vitro* immune response cultures, the anti-Ia MAb inhibits the proliferative response to bovine insulin and poly ($Glu^{50}Tyr^{50}$). In studies on mitogenesis it was found that the anti-Ia MAb inhibited the response to LPS but not to concanavalin A or phytohaemagglutinin. The effect of the anti-Ia MAb on other mitogens was found to vary from rabbit to rabbit.

Abbreviations: MHC, major histocompatibility complex; MLR, mixed lymphocyte response; MAb, monoclonal antibodies; MLN, mesenteric lymph node cells; PBL, peripheral blood lymphocytes; SDS, sodium dodecyl sulphate; FITC, fluorescein isothiocyanate; Ig, immunoglobulin; RITC, tetramethylrhodamine isothiocyanate; Con A, concanavalin A; PHA, phytohaemagglutinin; PWM, pokeweed mitogen; LPS, lipopolysaccharide; DS, dextran sulphate; NWSM, nocardia water soluble mitogen; LC, light-chain; c.p.m., counts per minute; MLC, mixed lymphocyte culture; SDS-PAGE, SDS-polyacrylamide gel electrophoresis; Ir, immune response; T cell, thymus-derived lymphocyte; B cell, bone marrow-derived lymphocyte; PPD, partially purified derivative; KLH, keyhole limpet haemocyanin; Ag8, P3X63 Ag8.6.5.3. myeloma.

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INTRODUCTION

The major histocompatibility complex (MHC) consists of a series of closely linked genetic loci that control a number of important immunological functions (Klein, 1975). In the rabbit, two loci, RLA-A and RLA-D, have been identified within the MHC. The RLA-A locus codes for the production of serologically defined alloantigens analogous to the murine Class I antigens (Tissot & Cohen, 1972). The RLA-D locus codes for a strong mixed lymphocyte response (MLR) (Lancki, Tissot & Cohen, 1979). Recently, alloantisera were produced that recognize two Ia alloantigens controlled by allelic genes at a locus closely linked to the MHC (Knight, Leary & Tissot, 1980).

In other species, specific allo-Ia-antibodies have been used as tools for the analysis of the molecular structure and cellular function of Ia molecules. Inbred and congenic strains of rabbit have been difficult to develop and thus the production of Ia specific alloantisera has been hindered. In order to overcome these problems we have attempted to produce mouse monoclonal antibodies (MAb) against rabbit Ia antigens. We report here the preparation and characterization of a monoclonal antibody which detects Ia molecules. This antibody was shown to inhibit the

mixed lymphocyte response, the proliferative response *in vitro* to bovine insulin and poly (Glu^{50} Tyr^{50}), and the response of lymphocytes to some mitogens.

MATERIALS AND METHODS

Cell fusion and initial screening

BALB/c mice (Lab Supply Indianapolis, IN) were immunized weekly with 2×10^7 rabbit spleen cells by intraperitoneal injection. Four days after the last injection the mice were killed and their spleen cells were used for fusion with P3X63-Ag8.6.5.3 (Ag8) myeloma cells which do not secrete Ig heavy or light chains, as previously described (Kearney *et al.*, 1979). Initial screening for antibody production was performed by a radiobinding assay using a mixture of an equal number of rabbit spleen and mesenteric lymph node cells (MLN) (McNicholas *et al.*, 1981a). Supernatant fluid from hybridomas found to be secreting antibody to rabbit tissue, were then tested by indirect immunofluorescence on rabbit spleen and thymus cells (see below). Hybridomas found to be secreting antibody reacting with rabbit spleen cells but not with thymus cells were cloned by limiting dilution (Oi & Herzenberg, 1980). The immunoglobulin isotype of the hybridoma monoclonal antibodies were determined by Ouchterlony analysis using class specific anti-mouse isotype reagents.

Cell preparations

The rabbits used throughout these studies were obtained from the closed colony of Drs S. Dray and K. L. Knight at the University of Illinois at Chicago, Health Sciences Center. Homozygous Ia^1/Ia^1 and Ia^2/Ia^2 rabbits were obtained from established lines (Knight *et al.*, 1980). Lymphoid tissues were excised, rinsed in RPMI-1640 (Gibco, Grand Island, NY), and single cell suspensions were prepared. Peripheral blood lymphocytes (PBL) were isolated from heparinized blood by sedimentation in 5% dextran T500 Pharmacia, Piscataway, NJ) and subsequent Ficoll–Hypaque gradient centrifiguation (Gathings *et al.*, 1981). Cell preparations were generally greater than 90% viable as determined by trypan blue exclusion.

Cell labelling and immunoprecipitation

Rabbit spleen cells were biosynthetically labelled with [^{35}S]-methionine and lysed as previously described (Jones, 1980). Lysates were cleared of rabbit IgG by addition of *Staphylococcus aureus* Cowen I. Immunoprecipitations of cleared lysates were performed by standard conditions as previously described (Jones, 1980). In some experiments, however, the buffer used to wash the precipitate contained 0·1% SDS. The precipitates were reduced and alkylated and electrophoresed in 10% SDS-polyacrylamide slab gels (SDS-PAGE) for 18 hr at 30–40 mV per plate (Weber & Osborn, 1969). The gels were then fixed, impregnated with 20% 2,5diphenyloxazole in dimethylsulphoxide and autoradiographed on Kodak XAR-5 film with a Dupont Lightning Plus enhancing screen at -70° (Bonner & Laskey, 1974). The films were exposed for 2 and 7 days.

Membrane immunofluorescence

Supernatant fluids were reacted with 2×10^6 rabbit lymphoid cells for 30 min at 4°. The cells were then washed and stained with a fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse immunogloblin reagent (Goding, 1976). After an additional 30 min incubation at 4° the cells were washed and analysed. Immunoglobulin (Ig)-positive cells were identified with a tetramethyl rhodamine isothiocyanate (RITC)-conjugated goat anti-rabbit Ig reagent (Goding, 1976). Visual analysis was performed with a Zeiss Universal Fluorescent Microscope equipped with an epi-condenser III RS; between 200 and 300 cells were counted for each sample. Immunofluorescent analysis was also performed using a fluorescence activated cell sorter (EPICS V, Coulter, Hialeah, FL) and 10,000 viable mononuclear cells, as determined by forward angle light scattering, were analysed.

Cell culture

All cell cultures were performed using RPMI-1640 medium supplemented as previously described (Metzger *et al.*, 1977) except that 5% foetal calf serum was used. The mitogens used in these experiments were: concanavalin A (Con A), 1·25 μg/ml (Calbiochem, San Diego, CA); phytohaemagglutinin (PHA), 0·25 μl/ml (Difco, Detroit, MI); pokeweed mitogen (PWM), 8 μl/ml (Gibco); lipopolysaccharide (LPS), 50 μg/ml (Difco); dextran sulphate (DS), 50 μg/ml (Difco); nocardia water soluble mitogen (NWSM), 50 μg/ml (Bona, Damis & Chedid, 1974); and goat anti-rabbit light-chain (anti-LC), 50 μg/ml. Cells (2×10^5) were placed in microtitre plates (Falcon Micro Test III, Cockeysville, MD) with mitogen in a total volume of 0·1 ml. To this, 0·1 ml of control (Ag8) or MAb ascitic fluid was added and the plates incubated at 37° in 5% CO_2 for 72 hr. Eighteen hours

prior to harvesting, 1 μCi of [^{3}H]-thymidine (6·7 Ci/mmol) was added to each well. Cells were harvested onto glass fibre filters and the incorporated radioactivity was measured in a Packard Tricarb scintillation counter. The data are presented as the mean counts per minute (c.p.m.) of triplicate cultures; the standard deviation of the triplicates is not shown as it was less than 10% in all cases.

One-way mixed lymphocyte cultures (MLC) were performed in microtitre plates by adding 0·05 ml of responding cells (4×10^6/ml) and 0·05 ml of stimulating cells (8×10^6/ml, X-irradiated with 2100 rads) to 0·1 ml of control (Ag8) or MAb ascitic fluid. Cells were incubated at 37° in 5% CO_2 for 5 days. Eighteen hours before harvesting, 1 μCi of [^{3}H]-thymidine was added to each well. Radioactivity incorporated was measured as above.

Immune response cultures were performed by adding 0·05 ml of cells (4×10^6/ml) and 0·05 ml of either bovine insulin (Eli Lilly, Indianapolis, IN), or poly ($Glu^{50}Tyr^{50}$) (Miles, Yeda, Israel), KLH or PPD at 200 μg/ml to 0·1 ml of control (Ag8) or MAb ascitic fluid. Cells were incubated and harvested in a fashion similar to the MLR.

Immunizations

Rabbits were immunized with 250 μg of poly ($Glu^{50}Tyr^{50}$), bovine insulin or KLH antigen dissolved in Freund's complete adjuvant, by subcutaneous injection in the hind foot pads and the back of the neck. Twenty-one days later, the rabbits received a similar dose dissolved in saline via intraperitoneal injections. Cultures were performed 10–15 days later.

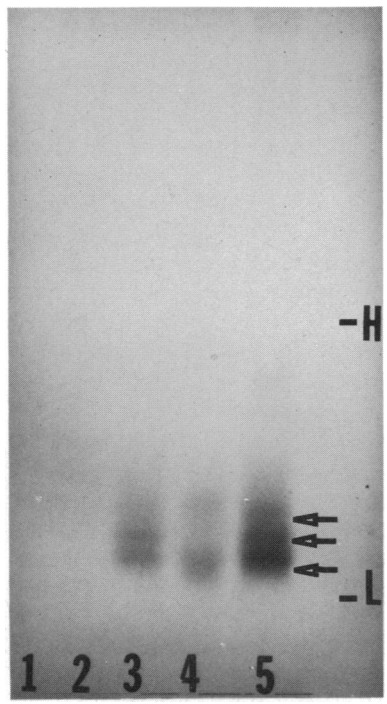

Figure 1. SDS-PAGE of immunoprecipitation of [^{35}S]-methionine-labelled spleen cell lysate from an Ia^1/Ia^2 heterozygous rabbit. Fresh lysate was immunoprecipitated by Ag8 control supernatant (Lane 1), normal rabbit serum (Lane 2), allo anti-Ia1 serum (Lane 3), allo anti-Ia2 serum (Lane 4), 2C4 MAb supernatant (Lane 5). *Arrows* indicate molecular weights of 35,000, 31,000 and 28,000 (top to bottom). H = immunoglobulin heavy chain, 55,000; L = immunoglobulin light chain, 25,000.

RESULTS

Identification of an Ia specific monoclonal antibody

Supernatant fluids from hybrid clones which reacted with rabbit spleen cells but not with thymocytes, were mixed with lysates from [^{35}S]-methionine-labelled spleen cells and the immunoprecipitates were analysed by SDS-PAGE. Analysis of the autoradiograms of the reduced immunoprecipitate obtained with the antibody from one clone, 2C4, revealed three bands corresponding to polypeptide chains of approximately 35,000, 31,000 and 28,000 (Fig. 1). These three bands were similar to those precipitated by alloanti-Ia sera (Knight *et al.*, 1980). The 31,000 band was found only when fresh lysates were used and the immunoprecipitates were washed by standard conditions. The 35,000 band was occasionally lost during the vigorous washing with 0·1% SDS. To establish if the 2C4 MAb reacted with rabbit Ia molecules, sequential immunoprecipitation analyses were performed with [^{35}S]-methionine-labelled spleen cell lysates prepared from Ia^2/Ia^2 or Ia^1/Ia^1 homozygous rabbit lines. Pretreatment of labelled spleen cell lysates from Ia^2/Ia^2 homozygous rabbits with anti-Ia2 alloantiserum removed all molecules which could react with the 2C4 MAb (Fig. 2). Likewise, pretreatment of the lysate with the 2C4 MAb removed all molecules capable of reacting with the anti-Ia2 alloserum (Fig. 2). In similar sequential radioimmunoprecipitation experiments with homozygous Ia^1/Ia^1 rabbits, the 2C4 MAb was shown to remove essentially all molecules which reacted with anti-Ia-1 alloantiserum and vice versa. Thus 2C4 MAb detects rabbit Ia molecules and

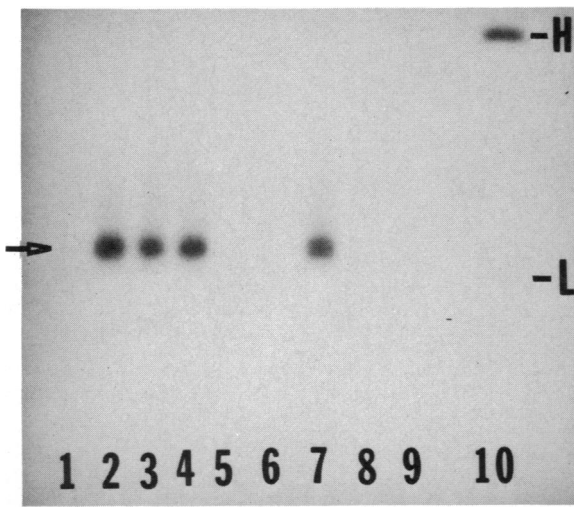

Figure 2. SDS-PAGE of sequential immunoprecipitation of [^{35}S]-methione-labelled spleen cell lysate from an Ia^2/Ia^2 homozygous rabbit. The lysate was divided into three aliquots, one aliquot was immunoprecipitated with normal rabbit serum (Lane 1); the supernatant from this immunoprecipitation was then re-immunoprecipitated with either an allo anti-Ia2 serum (Lane 2) or with the anti-Ia MAb, 2C4 (Lane 3). Another aliquot of the lysate was immunoprecipitated with the allo anti-Ia2 serum (Lane 4); the supernatant from this immunoprecipitate was then re-immunoprecipitated with either more allo anti-Ia2 serum (Lane 5) or with anti-Ia MAb, 2C4 (Lane 6). The third aliquot of the lysate was immunoprecipitated with the anti-Ia MAb, 2C4 (Lane 7); the supernatant from this immunoprecipitation was then re-immunoprecipitated with either more anti-Ia MAb, 2C4 (Lane 8) or with allo anti-Ia2 serum (Lane 9). The *arrow* indicates a molecular weight of 28,000. H = immunoglobulin heavy chain, 55,000; L = immunoglobulin light chain, 25,000 (Lane 10). Immunoprecipitates were washed in 0·1% SDS.

appears to react with all of the splenic Ia molecules of these rabbits. The 2C4 MAb has also been shown to immunoprecipitate the splenic Ia molecules of each of 15 randomly selected rabbits, indicating that the 2C4 MAb detects an isotypic determinant on all rabbit Ia molecules.

By Ouchterlony analysis the anti-Ia MAb supernatant fluid reacted only with antisera to mouse IgG2a, but not with antisera to IgM, IgA, IgG1, IgG2b or IgG3. Thus the anti-Ia MAb is of the mouse IgG2a class.

Distribution of Ia molecules on lymphoid cells

Lymphoid cells from the spleen, thymus, MLN, Peyer's patches, appendix, bone marrow and peripheral blood were analysed for the presence of Ia and Ig molecules by membrane immunofluorescence. In each of seven animals tested by one-colour immunofluorescence, the number of Ia-positive (Ia^+) cells, as detected by 2C4 MAb was similar to the number of Ig^+ cells (Table 1). The fluorescence profile as determined by laser flow cytometry of the anti-Ia MAb on various organs is shown in Fig. 3. Two populations of Ia^+ cells can be found in spleen, MLN, appendix, and Peyer's patches, a bright population consisting of 60–80% of the positive cells, and a dull population consisting of 20–40% of the positive cells. The fluorescent profiles of bone marrow and PBL, although displaying heterogeneity of fluorescent activity, do not segregate into two clear Ia^+ populations. In each of three two-colour membrane immunofluorescent experiments with the anti-Ia MAb and anti-rabbit LC antibody, essentially all of the fluorescent cells appeared as double stained Ig^+Ia^+ cells; a small percentage of cells however were Ig^+Ia^- and a few were Ig^-Ia^+ (Table 2). These results were similar to those previously obtained with anti-Ia alloantiserum (Knight *et al.*, 1980).

Inhibition of mixed lymphocyte response by anti-Ia MAb

In other species it has been shown that the MLR response can be inhibited by anti-Ia antibodies (Meo *et al.*, 1975). To determine if the anti-Ia MAb was capable of inhibiting this response, one-way MLR experiments were performed in the presence of anti-Ia MAb or control Ag8 ascitic fluid. In each of three

Table 1. Percentage of Ia and Ig bearing cells from various rabbit lymphoid organs

	Percentage positive cells from											
	Spleen		MLN		PBL		Peyer's patch		Appendix		Thymus	
	Ia	Ig	Ia	Ig	Ia	Ig	Ia	Ig	Ia	Ig	Ia	Ig
Rabbit												
1	39	38	36	34	29	30	42	39	61	58	2	2
2	42	39	38	37	61	58	43	42	60	62	2	2
3	56	56	40	42	57	56	52	52	59	60	3	1
4	57	53	49	47	41	45	59	57	80	81	4	3
5	35	37	51	51	32	31	55	56	67	65	6	5
6	37	32	44	40	40	37	67	66	56	54	3	4
7	41	38	32	30	30	29	65	61	51	49	2	3

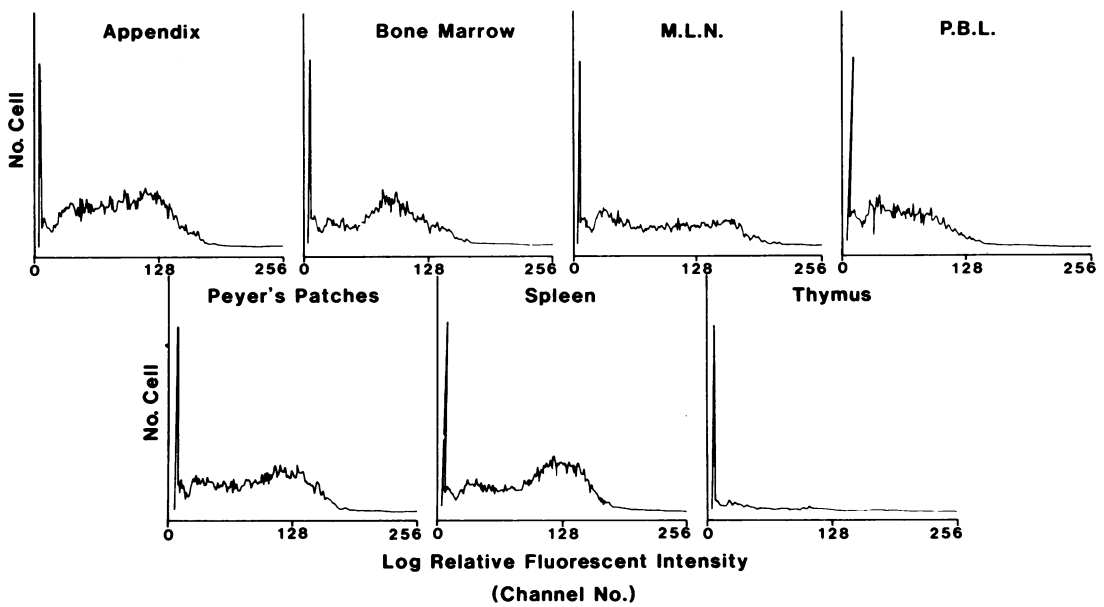

Figure 3. One parameter histograms of lymphocytes reacted with anti-Ia MAb, 2C4.

experiments, approximately 80% inhibition of the MLR was obtained. The inhibitory effect of the anti-Ia MAb ascitic fluid was observed to a final dilution of 1:16000 (Fig. 4).

The effect of anti-Ia MAb on immune responses *in vitro*

Antibodies to Ia molecules have been shown to inhibit the secondary *in vitro* proliferative response of mouse and guinea-pig lymphocytes to various natural and synthetic antigens (Shevach, Paul & Green, 1972; Schwartz *et al.*, 1978). To determine if the anti-Ia MAb would inhibit this response, spleen cells from rabbits that had been immunized with various antigens were cultured in the presence of anti-Ia MAb and antigen. The *in vitro* proliferative response to bovine insulin and poly ($Glu^{50}Tyr^{50}$) was inhibited approximately 40–90% by the anti-Ia MAb (Fig. 5). Similar results were obtained in two additional experiments with bovine insulin and poly ($Glu^{50}Tyr^{50}$). In contrast, the

Table 2. Two-colour membrane immunofluoresence of rabbit lymphoid cells with anti-Ia MAb and anti-LC antibody

Rabbit	Tissue	Percent cells		
		Ig^+Ia^+	Ig^-Ia^+	Ig^+Ia^-
1	Spleen	26	3	4
2	Spleen	31	6	3
	PBL	31	7	3
	MLN	38	2	2
3	Spleen	30	5	5
	PBL	70	3	10
	MLN	30	3	5
	Thymus	1	5	2

anti-Ia MAb did not inhibit the secondary *in vitro* proliferative response of rabbit lymphocytes to KLH or PPD (data not shown).

Effect of anti-Ia MAb on mitogenesis

To determine the effect of the anti-Ia MAb on mitogenesis, splenic lymphocytes were cultured in the presence of anti-Ia MAb and mitogen. In each of six experiments the anti-Ia MAb inhibited by 30–60% the response to the B cell mitogen, LPS (Table 3). The response to the B cell mitogens DS, NWSM, anti-LC and PWM was variable in that the response to each mitogen was partially inhibited in some, but not all, animals. For example, the response to DS was inhibited by 45–60% in four of six experiments, but by less than 21% in the remaining experiments. Furthermore, the pattern of inhibition of mitogenesis was different in the various rabbits; for example, whereas the anti-Ia MAb partially inhibited the response to LPS, DS and PWM in rabbit No. 3, it essentially did not inhibit the response to anti-LC or NWSM in the same rabbit (Table 3). The anti-Ia MAb had no affect on the response to T cell mitogens.

DISCUSSION

We have isolated a monoclonal antibody, 2C4, that detects rabbit Ia molecules. Sequential immunoprecipitation experiments have shown that the Ia molecules detected by the anti-Ia MAb are identical to the Ia molecules identified with alloanti-Ia sera. Furthermore, the anti-Ia MAb detected Ia molecules in every rabbit tested, regardless of their Ia phenotype. These results suggest that the anti-Ia MAb detects an isotypic determinant present on all rabbit splenic Ia molecules. Although at the present time we do not know whether this MAb reacts with α and/or β chains, we believe it may be reacting with β chains since washing of the immunoprecipitate with SDS results in the loss of the α chain from the complex.

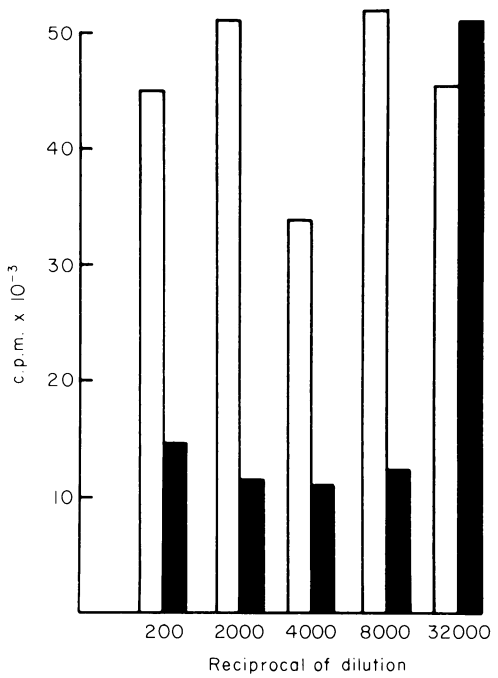

Figure 4. Inhibition of the MLR by anti-Ia MAb. Responder and stimulator cells were cultured in the presence of various dilutions of Ag8 control ascitic fluid (□) or anti-Ia MAb ascitic fluid (■).

Two-colour membrane immunofluorescent staining of rabbit lymphocytes from various organs has shown that the anti-Ia MAb detected most of the Ig^+ (B) cells. The small number of Ia^-Ig^+ cells may represent either a distinct population of B cells lacking Ia or, more likely, a population of B cells with a density of Ia too low to be visually detected. This latter possibility is supported by one-colour fluorescence-activated cell sorter analysis of anti-Ia MAb-treated spleen cells. When such cells are examined, two populations of cells can be seen (Fig. 3), one population, consisting of the majority of positive cells, is very brightly stained and a second population, consisting of only a few percentage

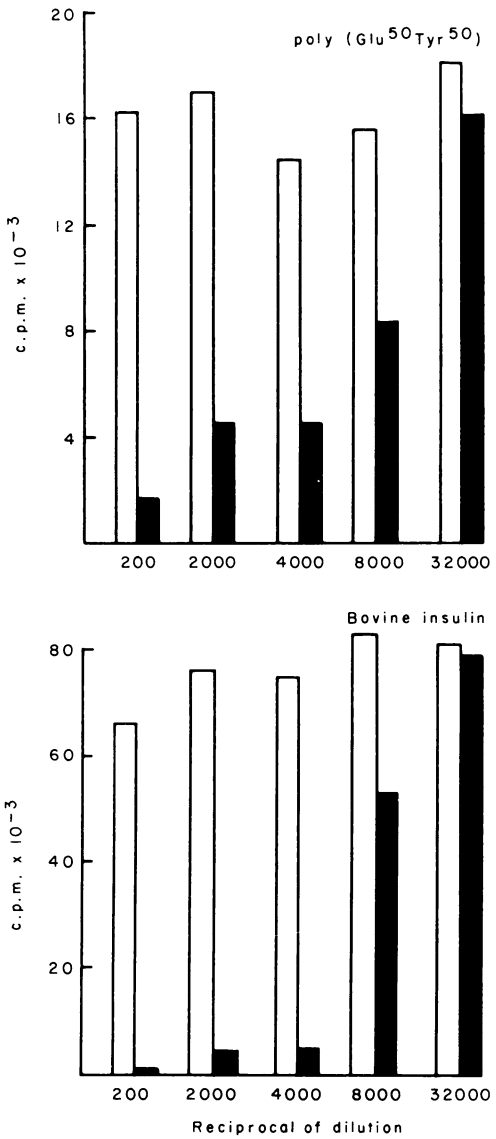

Figure 5. Inhibition of the secondary proliferative response *in vitro* to poly ($Glu^{50}Tyr^{50}$) or bovine insulin by anti-Ia MAb. Spleen cells from primed rabbits were cultured in the presence of antigen and varying dilutions of either Ag8 control ascitic fluid (□) or anti-Ia MAb ascitic fluid (■).

of the positive cells, is weakly stained. This weakly stained population may perhaps escape the less sensitive visual analysis, thus accounting for the few Ia^-Ig^+ cells seen in the visually analysed two-colour fluorescence.

The role of Ia^+ cells in immune functions, were established in a series of experiments in which the anti-Ia MAb was found to inhibit various immune functions. This inhibition was specific since non-anti-Ia mouse anti-rabbit MAb, prepared in this laboratory, had no effect on these responses (data not shown).

Addition of the anti-Ia MAb to mixed lymphocyte cultures significantly inhibited the mixed lymphocyte response (80% inhibition). This is consistent with results found in other species in which it has been shown that the Ia molecules are the stimulating determinants in the MLR, and that Ia antibodies inhibit the reaction (Meo *et al.* 1975).

In secondary *in-vitro* immune response cultures, the anti-Ia MAb significantly inhibited the proliferative response to both bovine insulin and poly ($Glu^{50}Tyr^{50}$). The ability to inhibit the response to bovine insulin and poly ($Glu^{50}Tyr^{50}$) demonstrates a role for Ia molecules in the response to these antigens. In contrast to these results, the anti-Ia MAb did not inhibit the response to KLH or PPD. The inability to inhibit the response to KLH and PPD, however does not preclude a role for the Ia molecules in the response to these antigens. In other species, alloanti-Ia sera directed against multiple Ia specificities inhibit the antigen response *in vitro* to antigens under specific Ir gene control, as well as to antigens not under unigenic control (Ruhl & Shevach, 1975). In contrast, monospecific alloanti-Ia sera or anti-Ia MAb directed against a single Ia specificity inhibited only the response to antigens under specific Ir gene control (Shevach *et al.*, 1977; Baxevanis *et al.*, 1980; Ishii *et al.*, 1981; Huber *et al.*, 1982). The ability of an antibody to inhibit the immune response *in vitro* may be due to specific blocking of the epitope required for the response or possibly to steric hindrance of that epitope by antibody bound to an adjacent epitope. Thus it is quite reasonable to expect inhibition of responses *in vitro* by Ia antibodies directed to the specific epitope responsible for the response or to Ia determinants spatially close to that epitope. Ia antibodies directed to distant determinants might not interfere with the response (Benacerraf, 1978). Thus, the response to simple antigens such as poly ($Glu^{50}Tyr^{50}$) and bovine insulin which may require only a single Ia epitope are easily inhibited by specifically blocking or sterically hindering the epitope. On the other hand, the response to complex antigens such as KLH and PPD which may interact with multiple Ia epitopes is difficult to block; blocking one epitope by an anti-Ia MAb may not

Table 3. Effect of anti-Ia MAb on mitogenesis

Rabbit	MAb	Mitogen*						
		Con A	PHA	PWM	LPS	Anti-LC	DS	NWSM
1	Control	143227	91831	50253	14010	2047	22593	6595
	Ia	135779	94315	**40185**	**7225**	**851**	**12577**	4323
2	Control	164937	127447	68627	16954	17758	26079	8457
	Ia	179714	131261	62112	**8340**	**7615**	**10946**	**4271**
3	Control	159676	138684	69124	12267	62413	29087	5849
	Ia	161611	139734	**50246**	**4745**	62549	**14686**	5114
4	Control	152199	130938	61951	38259	12879	34373	18366
	Ia	131566	124019	58248	**22075**	**9033**	26959	16774
5	Control	154339	116958	76485	6352	2188	16982	7611
	Ia	168385	125887	67703	**3531**	**1214**	14460	**2662**
6	Control	180883	116144	36214	4321	5188	11126	3640
	Ia	151528	109035	**25689**	**2612**	5400	**6327**	**2419**

* Mean c.p.m. of triplicate; bold numbers were significantly less than control cultures ($P < 0{\cdot}05$).

necessarily hinder other epitopes responsible for the collective response.

Addition of the anti-Ia MAb to rabbit spleen cell cultures was found to inhibit the response to LPS in all rabbits, but only variably inhibit the response to the other B cell mitogens. Although LPS is a complex molecule it is essentially a single repeating determinant on a lipid backbone and may act as a simple antigen. Thus in a fashion similar to bovine insulin and poly ($Glu^{50}Tyr^{50}$), the response to LPS in all rabbits may be related to a single Ia epitope which is specifically blocked or sterically hindered by the anti-Ia MAb. The other B cell mitogens are complex molecules presumably with multiple determinants. Thus, similar to responsiveness to KLH and PPD, the response to these B cell mitogens may be related to multiple Ia epitopes. Some rabbits may have an epitope which can be affected by the anti-Ia MAb, thus blocking the response, while in other rabbits the epitope required for the ability to respond is not blocked. We cannot however, rule out the possibility that the inhibition was due to the close proximity of the mitogen receptors to the Ia molecules and interference of the binding of the mitogen by anti-Ia MAb. If this were the case, one would expect that all rabbits would have given similar patterns of mitogen responsiveness in the presence of the anti-Ia MAb since the anti-Ia MAb reacted with Ia molecules in all rabbits. Another possibility is that the anti-Ia MAb has some biological effect which alters the ability of cells to respond to mitogens. However, if this were the case, again we would have expected this to occur in all rabbits, since the anti-Ia MAb reacted with Ia molecule in all rabbits. Thus, we believe that the inhibition of mitogenesis is due to an interference of specific or spatially close Ia determinants by the anti-Ia MAb. This is the first study to show inhibition of B cell mitogen responsiveness by anti-Ia antibody. The relationship between Ia molecules and LPS responsiveness is presently being investigated.

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REFERENCES

Baxevanis C.N., Wernet D., Nagy Z.A., Maurer P.H. & Klein J. (1980) Genetic control of T-cell proliferative responses to poly($Glu^{40}A1a^{60}$) and poly ($Glu^{5}lys^{34}Tyr^{15}$): subregion specific inhibition of the responses with monoclonal Ia antibodies. *Immunogenetics*, **11**, 617.

Benacerraf B. (1978) A hypothesis to relate the specificity of T lymphocytes and their activity of I region-specific Ir genes in macrophages and B lymphocytes. *J. Immunol.* **120**, 1809.

BONA C., DAMIS C. & CHEDID L. (1974) Blastic transformation of mouse spleen lymphocytes by a water soluble mitogen extracted from Nocardia. *Proc. natn. Acad. Sci. U.S.A.* **71,** 1602.

BONNER W.M. & LASKEY R.A. (1974) A film detection method for tritium labelled proteins and nucleic acids in polyacrylamide gels. *Eur. J. Biochem.* **46,** 83.

GATHINGS W.E., MAGE R.G., COOPER M.D., LAWTON A.R. & YOUNG-COOPER G.O. (1981) Immunofluorescence studies on the expression of VHa allotypes by pre-B and B cells of homozygous and heterozygous rabbit. *Eur. J. Immunol.* **11,** 200.

GODING J.W. (1976) Conjugation of antibodies with fluorochromes: modification to the standard methods. *J. immunol. Meth.* **13,** 215.

HUBER T.B., HANSEN T.H., SKELLY R.R. & THORLEY-LAWSON D.A. (1982) IaW39 expression correlates with a specific Ir function. *J. Immunol.* **128,** 2349.

ISHII N., BAXEVANIS C.N., NAGY Z.A. & KLEIN J. (1981) Selection of H-2 molecules for the context of antigen recognition by T lymphocytes. *Immunogenetics*, **14,** 283.

JONES P.P. (1980) Analysis of radiolabelled lymphocyte proteins by one- and two-dimensional polyacrylamide gel electrophoresis. In: *Selected Methods in Cellular Immunology* (eds B. B. Mishell and S. M. Shiggi), p. 398. W.H. Freeman and Co., San Francisco.

KEARNEY J.F., RADBRUSH A., LIESEGANG B. & RAJEWSKY K. (1979) A new mouse myeloma cell line that has lost immunoglobulin expression but permits the construction of antibody secreting hybrid cell lines. *J. Immunol.* **123,** 1548.

KLEIN J. (1975) *Biology of the Mouse Histocompatibility 2 Complex*. Springer-Verlag, New York.

KNIGHT K.L., LEARY A.L. & TISSOT R.C. (1980) Identification of two Ia-like alloantigens on rabbit B lymphocytes. *Immunogenetics*, **10,** 443.

LANCKI D.W., TISSOT R.G. & COHEN C. (1979) Histocompatibility in the rabbit. Genetic control of rabbit mixed lymphocyte culture reactivity. *Transplant.* **27,** 79.

MCNICHOLAS J.M., RAFFELD M., LOKEN M.R., REITER H. & KNIGHT, K.L. (1981a) Monoclonal antibodies to rabbit lymphoid cells: preparation and characterization of a T-cell specific antibody. *Molec. Immunol.* **18,** 815.

MCNICHOLAS J.M., WATKINS J.R., JOHNSON A.D. & KNIGHT K.L. (1981b) Functional studies of rabbit T lymphocytes. *Immunology*, **43,** 635.

MEO T., DAVID C.S., RIJNBEEK A.M., NABHOLZ M., MIGGIANO V.C. & SHREFFLER D.C. (1975) Inhibition of mouse MLR by anti-Ia sera. *Transplant. Proc.* **7,** 127.

METZGER D.W., HENDRICKS R.L., TEODORESCU M. & DRAY S. (1977) Primary *in vitro* antibody response of rabbit lymphoid cells and T-B cell collaboration in the absence of detectable mitogens. *Cell. Immunol.* **32,** 33.

OI V.T. & HERZENBERG L.A. (1980) Immunoglobulin producing hybrid cell lines. In: *Selected Methods in Cellular Immunology* (eds B. B. Mishell and S. M. Shiggi), p. 351. W.H. Freeman and Co. San Francisco.

RUHL H. & SHEVACH E.M. (1975) The effect of alloantisera on antigen-induced T cell proliferation. *J. Immunol.* **115,** 1493.

SCHWARTZ R.H., DAVID C.S., DORF M.E., BENACERRAF B. & PAUL W.E. (1978) Inhibition of dual Ir gene-controlled T-lymphocyte proliferative response to poly (Glu^{56}-$Lys^{35}Phe^{9}$)n with anti-Ia antisera directed against products of either I-A or I-C subregions *Proc. natn. Acad. Sci. U.S.A.* **75,** 2387.

SHEVACH E.M., LUNDQUIST M.L., GECZY A.F. & SWARTZ B.D. (1977) The guinea pig I. region. II. Functional analysis. *J. exp. Med.* **146,** 561.

SHEVACH E.M., PAUL W.E. & GREEN I. (1972) Histocompatibility linked immune response gene function in the guinea pig. Specific inhibition of antigen induced lymphocyte proliferation by alloantisera. *J. exp. Med.* **135,** 1207.

TISSOT R.G. & COHEN C. (1972) Histocompatibility in the rabbit. *Tissue Antigens*, **2,** 267.

WEBER K. & OSBORN M. (1969) The reliability of molecular weight determinations by dodecylsulfate-polyacrylamide gel electrophoresis. *J. biol. Chem.* **244,** 4406.