ANTI-Ia ANTIBODY IN THE SERA OF NORMAL SUBJECTS AFTER IN VIVO ANTIGENIC STIMULATION*

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Although human Ia antigens were first recognized on B lymphocytes and macrophages (1, 2), recent studies have revealed that Ia antigens are also expressed on normal T cells (3–5), Langerhans cells (6), and some malignant cells (7, 8). A central role for Ia antigens in the regulation of immune responsiveness is suggested by the observations that antibodies to Ia inhibit antigen presentation (9) and bind soluble T cell-derived immunoregulatory factors (10–13). Ia-positive cells have been reported to be necessary in autologous and allogeneic mixed lymphocyte reactions (auto-MLR and allo-MLR)[1] (14) and to play a significant role in suppressor cell functions (15, 16).

In some presumed autoimmune diseases, such as rheumatoid arthritis and systemic lupus erythematosus (SLE) (17) as well as in infectious mononucleosis (18) and graft-vs.-host reaction (19), an increase in peripheral blood Ia-positive T cells has been recorded. Yu et al. (17) have reported that the percentage of peripheral blood Ia-positive T cells in normals increases after tetanus immunization or skin testing with purified protein derivative (PPD). In the present report, we also found an elevation of Ia-positive T cells in three of six immunizations in vivo, whereas, in the remaining three individuals studied, a slight decrease was observed. A more consistent finding, however, was the production of IgM anti-Ia-reactive antibodies after in vivo immunization or antigenic stimulation. These naturally occurring anti-Ia antibodies might regulate the percentage of Ia-positive T cells in peripheral blood.

Materials and Methods

Preparation of Lymphocytes. Peripheral blood mononuclear cells (PBMC) were separated from heparinized blood of normal donors by differential centrifugation over Ficoll-Hypaque. Purified T cells were obtained by incubation of PBMC with neuraminidase-treated sheep erythrocytes (SRBC) at 4°C for 1 h with subsequent Ficoll-Hypaque gradient centrifugation. The SRBC obtained in the rosetting fraction were lysed with ammonium chloride-Tris buffer. After washing, adherent cells were removed by incubation at 37°C for 45 min on glass petri dishes. Such T cell preparations were assayed for purity (>97%) using immunofluorescence and mouse

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[1] *Abbreviations used in this paper:* allo-MLR and auto-MLR, allogeneic and autologous mixed lymphocyte reactions; BSA, bovine serum albumin; ELISA, enzyme-linked immunosorbent assay; FACS, fluorescence-activated cell sorter; FCS, fetal calf serum; NHS, normal human serum; PBS, phosphate-buffered saline; PLL, poly-L-lysine; PPD, purified protein derivative; SLE, systemic lupus erythematosus; SRBC, sheep erythrocytes.

hybridoma antibody 9.6 reacting with E-rosette receptors (20). In addition, T cell preparations showed <1% cells with surface immunoglobulins (Ig) and <2% peroxidase-positive cells.

Monoclonal Antibodies. Mouse hybridoma anti-Ia monoclonal antibody (clone L243; B-D Immunodiagnostics, Becton, Dickinson & Co. Orangeburg, NY) was used. This reagent reacts with the common or core determinant of human Ia antigen and precipitates 28,000 and 34,000 mol wt chains from ^{125}I-labeled NP-40 extracts of human B cell lines (21).

Monoclonal Antibody Rosetting Technique for Determination of Ia-positive T Cells. The precise methods of the rosetting technique have been previously described (22). Briefly, T cells were incubated with anti-Ia monoclonal antibody for 30 min at 4°C, then washed twice with phosphate-buffered saline (PBS) and mixed with affinity-purified goat anti-mouse IgG (Tago Inc., Burlingame, CA) coupled by chronic chloride treatment to ox erythrocytes. The mixture was centrifuged for 10 min at 200 *g*, incubated for 30 min on ice, and the percentage of rosetting cells counted.

Measurement of Density of Ia Antigen on Cells Using the Fluorescence-activated Cell Sorter (FACS). E-rosette-negative cells were analyzed for Ia antigen by reacting with fluorescein-conjugated monoclonal anti-Ia antibody (Becton, Dickinson & Co.) with subsequent measurement of fluorescence intensity on an FACS-III (B-D FACS systems; Becton, Dickinson & Co.). A cell pellet containing 10^6 cells was incubated for 30 min on ice with 50 μl of fluoresceinated monoclonal anti-Ia (20 mg/ml). The cells were washed twice in a refrigerated centrifuge with PBS containing 0.01% azide and then were suspended in 1% paraformaldehyde-PBS and stored at 4°C. Cells obtained before and at days 1 through 7 after skin testing were all analyzed on the same day.

Blocking of Anti-Ia Monoclonal Antibody-binding by Serum Samples. The precise methods have also been described in our previous report (22). Briefly, T cells were preincubated with 100 μl of test serum samples for 1 h at 4°C, followed by incubation with appropriate dilutions of anti-Ia monoclonal antibody in the presence of serum samples. After sensitization, cells were washed, and the percentage of positive cells was analyzed using the rosette technique. The capacity of serum samples to block the binding of anti-Ia monoclonal antibody was assessed and reported as percent inhibition. In our previous report (22) on anti-Ia blocking activity in the sera of patients with SLE, we demonstrated that the rosette assay described above correlated well with the results of a similar analysis using an FACS. We also showed that the blocking activity was not reduced by pepsin digestion or ultracentrifugation, ruling out the possibility that the blocking activity was the result of immune complexes.

Anti-Ia-reactive Antibody Detected by ELISA Assay. An Ia-positive B cell line, B35 M, was used as a target cell substrate. 50 μl of poly-L-lysine (PLL) (Sigma Chemical Co., St. Louis, MO) (50 μg/ml in PBS) was placed in flat-bottomed flexible microtiter plates (Dynatech Laboratories Inc., Dynatech Corp., Alexandria, VA). Plates were incubated for 1 h at 37°C. After incubation, the wells were washed twice with PBS, and B35 M cells (5×10^4 cells/well) were added to each well with adherence to the bottom of the wells after centrifugation at 1,500 rpm for 10 min. After centrifugation, wells were washed once with PBS, and the remaining binding sites in the wells were blocked with 100 μl of 50% fetal calf serum (FCS) and incubated 2 h at room temperature.

After incubation, the wells were washed three times with PBS; 25 μl of serum samples was added to the wells in triplicate and incubated 1 h at room temperature, and then 25 μl of monoclonal anti-Ia antibody (4 μg/ml in PBS + 10% FCS) was added to the wells without washing (final concentration of monoclonal anti-Ia antibody was 2 μg/ml). The microtiter plates were then incubated for 30 min at room temperature, and wells were washed three times with PBS. After washing, 50 μl of peroxidase-conjugated, affinity-purified goat anti-mouse IgG (Tago Inc.) was added (1:1000 dilution in PBS with 10% FCS) and incubated at 4°C for 30 min. After washing, 100 μl of the substrate, 0.02% 2.2-azino-di-3-athylbenzthiazolin-sulfonate) in 2.3% citric acid buffer (pH, 4.0) with 0.17% hydrogen peroxide was added to the wells. After 30 min incubation at room temperature, the reaction was stopped by adding citric acid buffer, pH 2.8. The light absorbance of wells was measured by an automated ELISA reader (Dynatech Laboratories, Inc., Dynatech Corp.) To construct a standard curve, different final concentrations of monoclonal anti-Ia antibody were added to the wells in the presence of normal human serum (NHS) or FCS (Fig. 1). In preliminary experiments, different numbers of B35 M cells

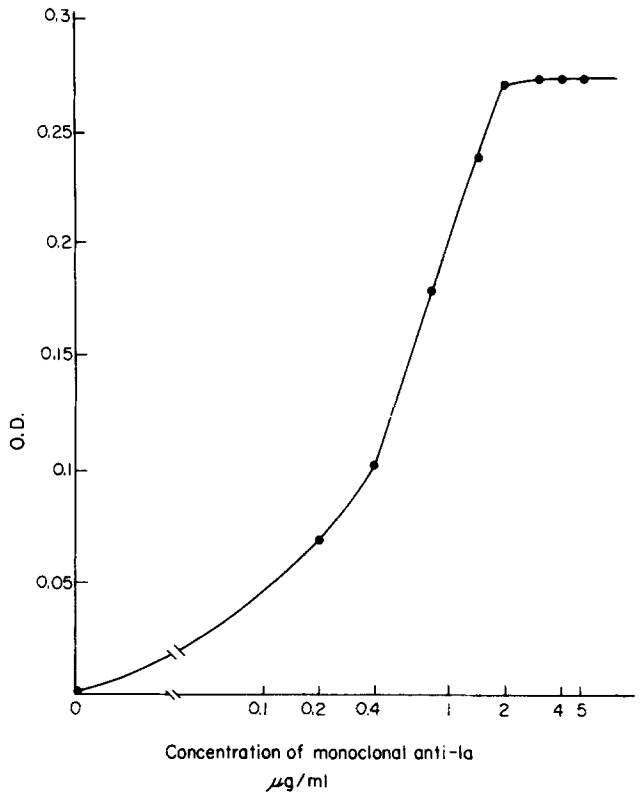

FIG. 1. Standard curve of binding of monoclonal anti-Ia antibody to microtiter wells coated with B35 M cells. 50,000 B35 M cells, an Ia-positive cell line, were adhered to microtiter plates with poly-L-lysine. Different qualities of anti-Ia antibody were incubated in the wells, then washed, and affinity-purified, peroxidase-coupled goat anti-mouse IgG was added. After washing, substrate was added, and the optical density at 410 nM was measured. In other experiments, like that in Fig. 4, a concentration of 2 μg/ml of the monoclonal anti-Ia antibody was used, and the ability of various sera samples to block the binding was measured.

were adhered to the plates. There was a linear increase in binding of monoclonal anti-Ia antibody with increasing numbers of B35 M cells up to 5×10^4 cells/well, above which there was little additional increase in binding of anti-Ia.

Detection of Anti-Ia Antibodies by ELISA Using Purified Ia Antigen. Ia antigens were purified from B35 M cells by a method modified from that of Winchester et al. (23). Briefly, 15 ml of packed B35 M cells were lysed with freeze-thawing and hypotonic washing. The membrane preparation was solubilized with 5% sodium deoxycholate, and the preparation was centrifuged at 100,000 *g* for 1 h. The supernatant was passed over an ultragel A 34 column equilibrated with 0.01 M Tris-HCl (pH, 8.0), 0.15 M NaCl, 0.001 M EDTA, and 0.1% deoxycholate. The eluted aliquots were assayed by enzyme-linked immunosorbent assay (ELISA) technique using rabbit IgG anti-Ia antibody coated to plates, followed by the antigen fractions and subsequent addition of mouse monoclonal anti-Ia conjugated with peroxidase. Fractions with high activity were pooled and subjected to 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. 2-mm slices of the gels were eluted with PBS plus 30% FCS and tested for activity by the ELISA assay. Gel isolation and assay confirmed that human Ia antigenic materials represented 28,000- and 34,000-mol wt bands.

100 μl of Ia antigen (3 μg/ml in PBS) was placed in flat-bottomed flexible microtiter plates (Dynatech Laboratories Inc., Dynatech Corp.) Plates were incubated overnight at 4°C. After incubation, the wells were washed with PBS, and the remaining binding sites in the wells were

blocked by incubating with 100 μl of 2% bovine serum albumin-PBS (BSA-PBS) for 2 h at room temperature.

After incubation, the wells were washed three times with PBS, and 100 μl of diluted serum samples (20% in PBS-BSA) was added to the wells in duplicate and incubated 1 h at room temperature. After incubation, the wells were washed once with PBS-Tween 80 (0.05% Tween 80, 0.01% sodium azide) and twice with PBS, and then 100 μl of peroxidase-conjugated affinity-purified goat anti-human IgG and IgM (Tago Inc.) was added (1:500 dilution in PBS-BSA) and incubated 30 min at room temperature. The rest of the ELISA was carried out as described above. As a background, wells were established without Ia antigen.

To quantitate the amount of IgG and IgM bound to the wells, different amounts of IgG and IgM (1–200 ng) were added to the wells and incubated overnight at 4°C. In preliminary experiments, we examined the percentage of nonspecific binding of IgG and IgM to the wells using ^{125}I-labeled Ig and found that 55% to 65% of Ig adhere to the wells by overnight incubation at 4°C. We added peroxidase-conjugated goat anti-human IgG or IgM for the ELISA assay as described above. We then plotted a standard curve of IgG or IgM vs. light absorption to quantitate the amount of IgG and IgM bound to the wells coated with Ia.

Absorption of Serum Samples with Cell Lines. In some experiments, sera samples were absorbed with human cell lines known to express surface Ia antigen (B35 M, Molt-3, W1-L2, RPMI 4098) or to be Ia negative (Molt-4) (1, 24). 1-ml serum samples were incubated with 10^8 cells at 4°C for 2 h, and then the cells were removed by centrifugation.

Affinity Purification of IgG and IgM from Serum Samples. Rabbit anti-human IgG and anti-human IgM (Calbiochem, LaJolla, CA) were conjugated with Sepharose 4B by the method described by Porath et al. (25). Serum samples were passed over the column and absorbed IgG or IgM eluted using pH 3.0, 0.1 M glycine saline buffer followed by concentration by evaporation and dialysis against PBS.

Antigenic Stimulation of Normal Subjects with Purified Protein Derivative (PPD), Tetanus Toxoid, and Candida Antigen. Three normal individuals known to show positive delayed-type hypersensitivity to PPD were injected intradermally with 0.1 ml of PPD (Parke Davis and Co., Detroit, MI); two other individuals were injected intracutaneously with 0.02 ml of Candida antigens (Bioproducts Corp., Tempe, AZ), and one other subject was injected with 0.5 ml of subcutaneous tetanus toxoid (Upjohn Co., Kalamazoo, MI). Blood samples were collected before the immunization and at periodic intervals afterwards, and percentage of Ia-positive T cells as well as anti-Ia blocking activity of the serum samples was examined using the rosette inhibition technique or ELISA assays. As a control, two individuals known to be PPD negative received intradermal PPD, and serum samples were collected and analyzed as described above.

Results

Fig. 2 shows the inhibition of monoclonal anti-Ia binding to Ia-positive T cells by sera from six subjects before and on days 1 through 7 after skin testing or vaccination. In all cases, there was substantial inhibition of the binding of anti-Ia antibody by sera obtained after in vivo antigenic challenge. The inhibitory activity was normally present 48 h after challenge and showed a peak between 48 and 120 h. In all cases it was gone by day 7. These same sera caused no inhibition of the binding of several other monoclonal antibodies directed against T cells (OKT3) or T cell subsets (OKT4 and OKT8; data not shown). As a control, two individuals known to be PPD negative were also given PPD. Sera from these individuals had no effect on binding of monoclonal anti-Ia antibody to Ia-positive cells.

Also graphed in Fig. 2 are serial studies of the percentage of Ia-positive T cells in the peripheral blood of these same subjects. In three instances there was an increase in the percentage of Ia-positive T cells within 48 h of skin testing, which was followed by a fall. The decrease in Ia-positive T cells coincided with the appearance of anti-Ia blocking activity in the sera of these subjects. In the remaining three subjects there was no initial rise in Ia-positive T cells; there was, however, a >50% decrease in the

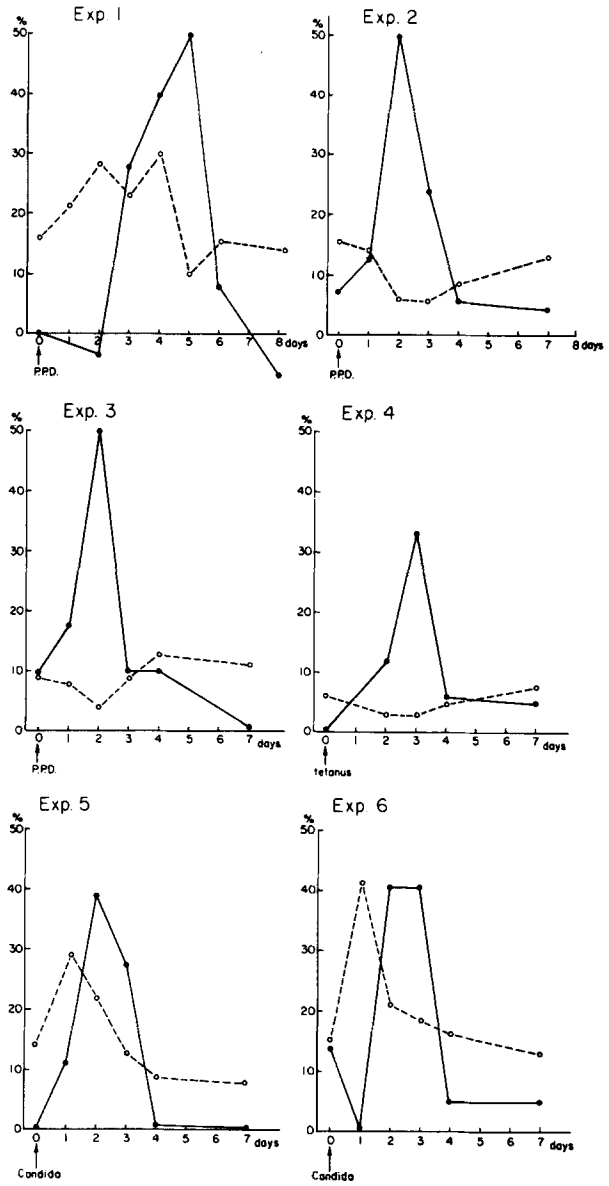

FIG. 2. Anti-Ia blocking activity in sera and percent Ia-positive T cells in blood of six individuals after in vivo antigenic challenge. Six normal volunteers were given either intradermal PPD (experiments 1–3), Candida antigens (experiments 5 and 6), or subcutaneous tetanus vaccine (experiment 4). In all cases, sera obtained between 2 and 4 d after immunization blocked the binding of monoclonal anti-Ia antibody to Ia-positive T cells. O, percent Ia-positive T cells; ●, percent inhibition of anti-Ia binding.

percentage of these cells occurring later in association with the appearance of anti-Ia blocking activity. In addition to a fall in the percentage of Ia-positive T cells in vivo, coincident with the appearance of the anti-Ia blocking activity in the sera, we also observed a decrease in demonstrable Ia antigen on non-T cells. Fig. 3 shows a dot plot

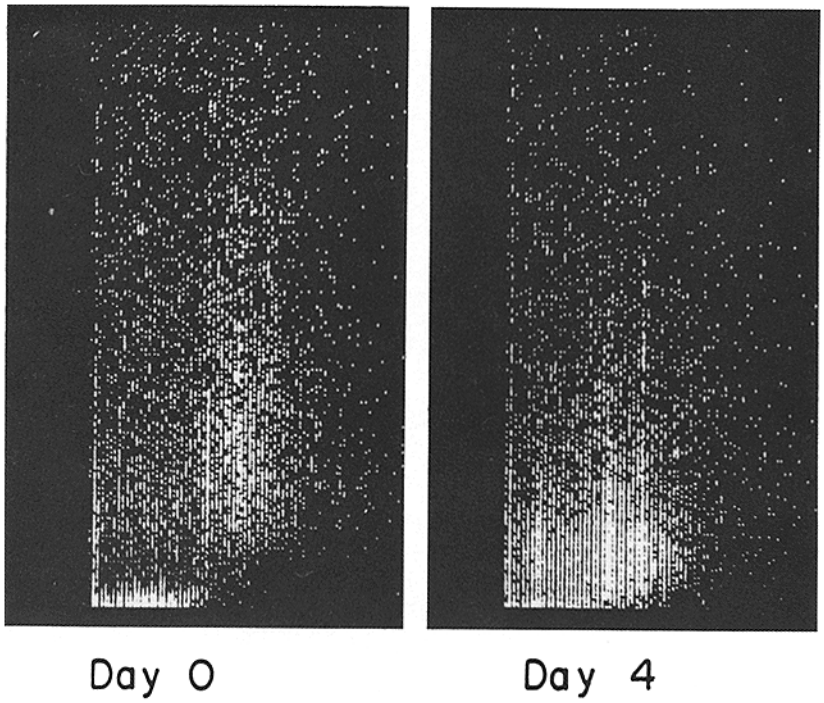

FIG. 3. FACS analysis of Ia-positive, E-rosette-negative cells before and 4 d after skin testing with PPD in a PPD-reactive subject. E-rosette-negative cells were reacted with fluorescein-conjugated monoclonal anti-Ia antibody and counted in the FACS. The vertical axis represents intensity of fluorescence, and the horizontal axis represents degree of light scatter, which correlates with cell size. The percent of Ia-positive cells was 59% on day 0 and 44% on day 4. In addition, on day 4 there was a marked diminution in fluorescence intensity in both small light-scatter cells (mostly B cells) and large light-scatter cells (mostly monocytes). The cell samples had been stored in 1% paraformaldehyde solution and were run at the same time without a change in the setting of the FACS. Cells from a PPD-negative individual obtained before and 4 d after PPD administration showed no change either in percentage of Ia-positive cells or intensity of fluorescence.

from the FACS of E-rosette-negative cells obtained before and 4 d after PPD administration stained with directly fluorescein-conjugated monoclonal anti-Ia. 59% of the non-T cells were Ia positive on day 0 vs. 44% on day 4. More impressive is the marked decrease in the fluorescence intensity after skin testing, suggesting that there was a decrease in density of surface Ia antigen on those cells that remained Ia positive. Similar data were obtained with another PPD-sensitive subject, whereas PPD administration to a PPD-negative individual resulted in no change in percentage of Ia-positive non-T cells or in intensity of staining.

We also measured anti-Ia blocking activity in sera by an ELISA method that used an Ia-positive human tumor cell adherent to microtiter wells. The blocking of monoclonal anti-Ia binding was assayed by preincubating the wells with test human sera. Fig. 4 presents such an analysis of anti-Ia blocking activity for the test subject shown in experiment 1, Fig. 2. Results of the two methods of measuring anti-Ia blocking activity were virtually identical.

We next examined which component of the sera from subjects after delayed-type hypersensitivity skin testing contained the anti-Ia blocking activity. In a preliminary

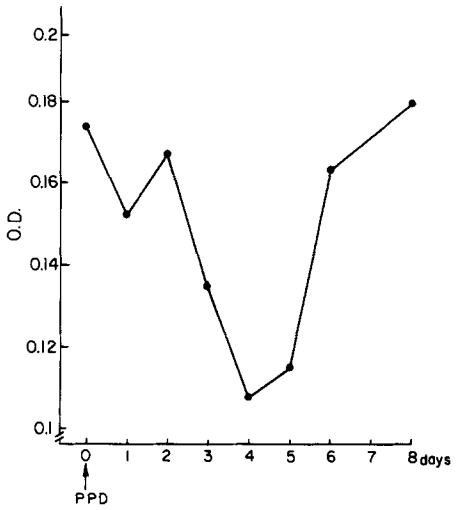

FIG. 4. Measurement of anti-Ia blocking activity after PPD challenge using an ELISA technique. Sera obtained before and at days 1, 2, 3, 4, 5, 6, and 8 after intradermal PPD injections were tested for their ability to block the binding of monoclonal anti-Ia antibody (2 μg/ml) to B35 M cells that had been adhered to microtiter wells. Binding of the monoclonal anti-Ia antibody was measured with affinity-purified, peroxidase-coupled goat anti-mouse IgG in an ELISA assay. There was a fall in optical density on days 3 to 5 after PPD administration, showing that the sera is blocking binding of the monoclonal anti-Ia. Calculating from the standard curve in Fig. 1, the optical density of 0.11 on day 4 represents a 63% inhibition of anti-Ia binding.

experiment using starch gel electrophoresis, we found that the blocking activity was contained in the gamma globulin fraction (data not shown). We therefore isolated the IgG and IgM fractions of three sera by affinity chromatography using rabbit anti-human IgG or IgM conjugated to Sepharose 4B. In each case, the blocking activity was contained entirely in the IgM fraction (Table I).

The data above suggested that blocking of monoclonal anti-Ia to Ia-positive cells was mediated by an IgM antibody. Table II provides evidence that this blocking is not genetically restricted. That is, sera from one subject blocked the binding of monoclonal anti-Ia antibody to Ia-positive T cells from subjects of several different DR haplotypes. These results suggested that the blocking activity could be caused by an IgM antibody directed against the common or core Ia determinants. To examine this directly, we absorbed sera with either an Ia-positive or Ia-negative human tumor cell line. As shown in Table III, absorption with B35 M cells (an Ia-positive line) eliminated the blocking activity of sera from subjects after skin testing, whereas absorption with Molt 4 cells (Ia negative) had no effect. Similar results were obtained with three other Ia-positive cell lines (experiment 4 in Table III).

The data presented above would appear to be strong though indirect evidence for the presence of IgM anti-Ia antibodies after in vivo immunization. We attempted to demonstrate this phenomenon more directly by using an ELISA assay in which Ia antigen purified from B35 M cells was adhered in microtiter wells, and the binding of IgG and IgM to these wells from the sera of subjects was measured before and after immunization. In addition, this method allowed us to roughly quantitate the amount of anti-Ia antibody in the sera. These results are shown in Table IV for three experiments on different donors. Before skin testing there was some baseline binding

TABLE I

Blocking of Anti-Ia Binding by Sera from Subjects after Skin Testing: Effect of Whole Sera and of IgG and IgM Fractions Isolated by Affinity Chromatography

Experiment	Days after skin test	Antigens	Immunoglobulin class	Percent anti-Ia inhibition
1	0	(-)	Whole serum	0%
			IgG	0%
			IgM	0%
1	5th	PPD	Whole serum	49.9%
			IgG	0%
			IgM	35.5%
4	3rd	Candida	Whole serum	33.0%
			IgG	0%
			IgM	43.5%
5	2nd	Candida	Whole serum	39.0%
			IgG	0%
			IgM	37.5%

100 μl of whole or 100 μl of affinity-purified IgG fractions of the sera were tested for their blocking activity. The IgG concentration was 3 mg/ml and the IgM was 1 mg/ml.

TABLE II

Blocking of Anti-Ia Binding by Sera from Subjects after Skin Testing: Sera from One Subject Blocks the Binding of Anti-Ia Antibody to Ia-positive T Cells from Three Donors of Different Ia Types

Source of Sera	Percent Ia-positive T cells			Mean inhibition
	Source of T cells			
	A(DR 3,7)	B(DR 4,6)	C(DR 1,5)	
Normal sera (B)	19	10	19	
Normal sera (C)	21	10	18	
Sera from subject A after skin testing:				
Day 0	22	12	23	0%
Day 1	24	12	22	−2%
Day 2	22	12	25	−4%
Day 3	12	10	17	28%
Day 4	14	7	13	40%
Day 5	12	6	10	50%
Day 6	28	9	16	8%
Day 8	24	12	26	−7%

Subject A was given intradermal PPD, and the ability of his sera on days 0 through 8 after PPD administration was tested for the ability to block binding of monoclonal anti-Ia to Ia-positive T cells that were autologous (subject A) or that were two other donors of different DR haplotypes (subjects B and C). Sera on days 3, 4, and 5 blocked the binding of monoclonal antibody to the three different target cells to a similar extent. The mean percent inhibition of monoclonal anti-Ia binding to the Ia-positive T cells from the three donors by the sera is given in the last column.

TABLE III

Anti-Ia Blocking Activity in Sera of Subjects after Skin Testing: Effect of Absorption with Ia-positive and Ia-negative Cell Lines

Experiment	Days after skin test	Absorbed with	Percent anti-Ia inhibition
1	4th	—	40.0%
		Molt-4	39.0%
		B-35 M	8.0%
2	2nd	—	51.0%
		Molt-4	37.0%
		B-35 M	4.0%
3	2nd	—	42.0%
		Molt-4	39.0%
		B-35 M	16.0%
4	3rd	—	24.0%
		Molt-4	29.0%
		B-35 M	0%
		Molt-3	9%
		WI-L2	5%
		RPMI 4098	5%

Sera from four subjects were tested for their ability to block monoclonal anti-Ia binding to Ia-positive T cells before and after absorption with Ia-negative (Molt-4) and Ia-positive (B-35 M, Molt 3, W1-L2, RPMI 4098) human cell lines. Percent inhibition by sera was calculated as compared with the amount of Ia-positive cells incubated in normal serum.

TABLE IV

Measurement of Anti-Ia Antibodies in Sera of Subjects before and after Delayed Hypersensitivity Skin Testing Using an ELISA Assay with Purified Ia Antigen

Inhibition	Time after skin test	Anti-Ia antibody		Percent anti-Ia
		IgM	IgG	
		μg/ml		
Experiment 1	Day 0	1.2	0.7	0%
(PPD)	Day 2	3.3	0.65	43%
Experiment 2	Day 0	0.8	0.9	7%
(Candida)	Day 4	2.5	1.0	57%
Experiment 3	Day 0	1.8	1.1	0%
(PPD)	Day 2	3.8	1.2	43%

Sera from three subjects taken before and after skin testing were tested for anti-Ia antibodies in an ELISA assay in which purified Ia antigen was adhered to microtiter plates. The sera after skin testing that gave the greatest amount of inhibition of monoclonal anti-Ia antibody to Ia-positive targets (right-hand column) were selected for testing. The amount of IgG and IgM bound to the wells was estimated by comparison to a standard curve. The experiments listed here are not the same as in Fig. 2.

of both IgG and IgM to the Ia-coated plates. After skin testing, the amount of IgM bound to the plates increased coincident with the appearance of anti-Ia blocking activity in the sera, whereas the amount of IgG anti-Ia was unchanged.

Discussion

We demonstrated that anti-Ia-reactive antibodies occur after in vivo antigenic stimulation in normal individuals. These antibodies were noted shortly after antigenic challenge, with the peak response occurring 2–5 d after immunization and disappearing by the 7th d. Anti-Ia antibodies occurring in normal subjects after in vivo antigenic stimulation were not HLA-DR restricted and reacted with Ia antigens on autologous and allogeneic T cells. Recently, Yu et al. reported that Ia-positive T cells increase after in vivo immunization (17). We also found an increase in Ia-positive T cells in three of the six subjects studied. A more constant finding was the occurrence of anti-Ia antibody, the peak of which coincided in all cases with a drop in Ia-positive T cells.

The physiologic functions of naturally occurring anti-Ia antibodies that are present in serum after antigenic stimulation in normal subjects are not presently known. In the autologous mixed lymphocyte response, it has been reported that T cells proliferate in response to autologous non-T cells (26, 27); an inhibitory effect of anti-Ia antibodies on this response has been reported from several laboratories (28–30). We recently reported (22)[2] that the sera of patients with active SLE contain anti-Ia antibodies of the IgG and IgM class. These anti-Ia antibodies blocked the binding of monoclonal anti-Ia antibodies to Ia-positive resting T cells, to activated T cells, and to non-T cell lymphocyte populations (22). Among the SLE patients studied, there was an inverse correlation between the amount of circulating anti-Ia antibodies and the percentage of Ia-positive T cells, suggesting a role for anti-Ia antibodies in the control of expression of Ia antigen on T cells. In addition, IgG anti-Ia antibodies in SLE sera, but not IgM anti-Ia antibodies, blocked the autologous MLR.[2] In preliminary studies, we could not find any effect on the autologous MLR of sera containing anti-Ia antibodies occurring after in vivo immunization in normals. This might be because the anti-Ia antibody in these subjects was IgM, a class of anti-Ia that in sera from SLE patients also did not affect the autologous MLR. Alternatively, it might be because of the small amount of anti-Ia antibody that we are measuring. Concentrations of monoclonal anti-Ia antibodies similar to the concentrations of the naturally occurring IgM anti-Ia antibody (1:5 dilution of 2 μg/ml; see Table IV) also do not inhibit the autologous MLR; higher concentrations are required (K. Okudaira, J. Goodwin, and R. C. Williams, unpublished data). It is also possible that naturally occurring anti-Ia antibodies might possess different functional effects according to their precise antigenic specificity. Supporting this point, Engleman et al. (30) reported different effects of different monoclonal anti-Ia antibodies on the autologous mixed lymphocyte response.

It is certainly unexpected that an antigen as widely expressed in vivo as is Ia could elicit an antibody response. It is also unclear why free anti-Ia antibody should exist in the sera in the presence of Ia(+) cells. It would appear that much of the anti-Ia did bind to Ia (+) T cells and non-T cells, resulting in a decrease in Ia(+) T cells and non-T cells and also in a decrease in the density of surface Ia on the non-T cells (Figs. 2 and 3). Other examples of free antibody circulating in the presence of endogenous

[2] Okudaira, K., R. Searles, J. Goodwin, and R. C. Williams, Jr. 1982. Antibodies against Ia antigen in the sera of patients with systemic lupus erythematosus block the autologous mixed lymphocyte response. *J. Immunol.* In press.

antigen include antilymphocyte antibodies in some autoimmune diseases and also the presence of antibodies against erythrocyte antigens in autoimmune hemolytic anemia.

Because antigen-reactive T cells recognize antigenic determinants in conjunction with self Ia antigens (31–33), anti-Ia antibodies occurring after in vivo immunization could conceivably influence antigen presentation by Ia-positive accessory cells. It is also possible that these naturally occurring anti-Ia antibodies could provide signals for specific antibody formation systems. In this regard, the anti-Ia antibody detected in the serum might represent a "leaking" of this antibody from the skin test site, and whatever function this anti-Ia antibody has might be restricted to the local site of the immune reaction. Although the naturally occurring anti-Ia antibodies described here after in vivo antigenic stimulation are not restricted to autologous HLA-DR phenotypes, these anti-Ia antibodies occur with specific antigen stimulation. This phenomenon is reminiscent of the idiotype-anti-idiotype network concept.

Summary

We showed that sera from normal subjects after antigenic challenge with intradermal PPD or Candida antigens or with subcutaneous tetanus vaccine contain a factor that blocks the binding of mouse monoclonal anti-Ia antibody to Ia-positive T cells or to B35 M cells, an Ia-positive human B cell line. The blocking activity appears 48 to 72 h after antigenic challenge and is gone by day 7. The appearance of the anti-Ia blocking activity coincided with a drop in the percentage of Ia-positive T cells and non-T cells in the peripheral blood of these subjects and also with a decrease in the density of surface Ia on the non-T cell population. The blocking was not genetically restricted; that is, serum from a given subject blocked anti-Ia binding to Ia-positive T cells of subjects with different DR haplotypes. The blocking activity was contained in the IgM fraction of the sera. The blocking activity of the sera was eliminated after absorption of the sera with Ia-positive but not with Ia-negative human cell lines. It would appear, therefore, that the blocking of monoclonal anti-Ia binding is caused by an IgM anti-Ia antibody that appears in normals after in vivo antigenic challenge.

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