IDIOTYPIC ANALYSIS OF LYMPHOCYTES IN VITRO

I. Specificity and Heterogeneity of B and T Lymphocytes Reactive with Anti-Idiotypic Antibody*

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Previous work from this laboratory has shown that passively administered anti-idiotypic antibody (anti-Id),[1] under certain experimental conditions, sensitizes mice to the antigen which is recognized by the corresponding idiotype (Id). This sensitization was shown to occur in T-helper as well as in B-precursor lymphocytes, suggesting that antigen receptors on both types of cells possess the same or similar idiotypes, and that the interaction of these receptors with anti-Id is functionally equivalent to that with antigen (1).

This work employed antibodies to Group A streptococcal carbohydrate (A-CHO), generated in strain A/J mice by immunization with Group A streptococcal vaccine (Strep.A) (2). A major portion of these antibodies represents a single antibody species which is defined by isoelectric-focusing spectrum (3) and by Id (2–4). The Id is detected by guinea pig anti-Id raised against the anti-A-CHO antibody produced by the A/J lymphocyte clone A5A (2–4). The expression of the A5A Id is controlled by a single gene within the Ig-1 complex of A/J mice (5). Anti-Id against the A5A Id can be fractionated into the IgG1 and IgG2 classes of guinea pig IgG (3, 6, 7). Whereas anti-Id belonging to the IgG2 class was shown to be suppressive (3, 6), stimulation of immunity was achieved with anti-Id of the IgG1 class (anti-A5A Id1) (1).

In this paper, we introduce an additional idiotypic system which employs a myeloma protein rather than an induced antibody for the production of anti-Id. The transplantable BALB/c plasmacytoma S117 secretes an IgA/k immunoglobulin with specificity for *N*-acetyl-glucosamine, the major antigenic determinant of A-CHO (8). Guinea pig anti-Id against this myeloma protein reacts with the antibodies of BALB/c mice immunized with Strep.A, and genetic analysis demonstrated that the expression of the S117 idiotypic marker is controlled by a gene within the Ig-1 complex of strain BALB/c (reference 9, and footnote 2). Unpublished experiments in vivo had indicated that passively administered anti-S117 Id of the IgG1 class (anti-S117 Id1) primed BALB/c mice to produce a secondary

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[1] *Abbreviations used in this paper:* A-CHO, Group A streptococcal carbohydrate; anti-Id, anti-idiotypic antibody; anti-Id1 and anti-Id2, anti-Id of the IgG1 and IgG2 classes of guinea pig IgG, respectively; BSS, balanced salt solution; C-CHO, Group C streptococcal carbohydrate; IBC, idiotype-binding capacity; Id, idiotype; NGPS, normal guinea pig serum; PFC, plaque-forming cells; Strep.A, Group A streptococcal vaccine; TNBS, 2,4,6-trinitrobenzene sulfonic acid; TNP, 2,4,6-trinitrophenyl; V, variable.

[2] Berek, C., and K. Eichmann. Manuscript in preparation.

anti-A-CHO response upon subsequent challenge with Strep.A, suggesting that induction of specific immunity had occurred.

Employing both these idiotypic systems for the in vitro analysis of lymphocytes primed with anti-Id, we investigated the specificity and the heterogeneity of Id-bearing T and B cells, as well as several possible objections raised against the interpretation of our previous in vivo experiments (1). The results suggest: (*a*) Antigen contamination is not responsible for the sensitization of lymphocytes with anti-Id. (*b*) Anti-Id produced against a myeloma protein sensitizes T-helper cells as effectively as does anti-Id against an induced antibody. This eliminates a possible role of antibodies to T-cell receptors in T-cell sensitization. (*c*) T and B lymphocytes primed with the same anti-Id possess specificity for the same carbohydrate determinant. (*d*) The restriction in the heterogeneity of the lymphocyte populations primed with anti-Id, as compared to that of lymphocytes primed with Strep.A, is similar for T and B cells. A brief account of these experiments has been previously published (10). Taken together, these data strongly suggest that the antigen recognition systems of B- and T-helper cells are alike with respect to heterogeneity, specificity, and idiotypic determinants. In the accompanying paper (11), we will present evidence that the same genes may participate in the coding for T- and B-cell receptor variable (V) regions.

Materials and Methods

Animals. 6- to 8-wk-old A/J mice were purchased from The Jackson Laboratory, Bar Harbor, Maine; and BALB/c mice were obtained from Zentralinstitut für Versuchstierzucht, Hannover, West Germany. All animals were vaccinated against ectromelia and rested at least 4 wk before being used in experiments. Guinea pigs were obtained from a local breeder.

Antigens. Sheep erythrocytes (SRBC) were purchased from Behringwerke, Marburg-Lahn, West Germany. Haptenation of SRBC with 2,4,6-trinitrobenzene sulfonic acid (TNBS) was performed by the method of Rittenberg and Pratt (12). The preparation of the stearoyl derivative of A-CHO and its coupling to SRBC were done by the method of Pavlovskis and Slade (13). Purified A-CHO and Group C streptococcal carbohydrate (C-CHO) were a gift from Dr. R. M. Krause, The Rockefeller University, New York.

Group A streptococci, strain J17A4, were also a gift from Dr. R. M. Krause and the preparation of the vaccine (Strep.A) was done as previously described (14). The haptenation of Group A streptococci with TNBS was achieved by a modification of the Rittenberg and Pratt technique (12). A 200 mg portion of TNBS was dissolved in 42 ml of cacodylate buffer. 10 ml of cacodylate buffer containing 1.5×10^{11} Strep.A particles were added under rapid mixing and the suspension was then stirred slowly at room temperature for 30 min. Coupled particles (TNP-Strep.A) were centrifuged for 10 min at 300 *g*, the supernate was decanted and replaced by 30 ml of cacodylate buffer containing 50 mg of glycyl-glycine. The particles were resuspended, centrifuged, and the supernate decanted as before. This procedure was followed by four washes in 50 ml of 0.15 M saline. The haptenation reaction was conducted sterilely in foil wrapped glass vessels.

Idiotypes and Anti-Idiotypic Antibodies. The preparation of antibody A5A from the sera of irradiated A/J mice reconstituted with cloned spleen cells has been described (2). Anti-Id antibodies to antibody A5A were prepared in guinea pigs and fractionated into their IgG1 and IgG2 classes by previously described methods (2, 3). The S117 myeloma protein was isolated from the sera or ascites of mice bearing myeloma tumor S117, using *p*-amino-phenyl-*N*-acetyl-glucosamine coupled to Sepharose 2B as an immunoadsorbent (15, 16). Anti-Id antibodies to S117 and their IgG1 and IgG2 fractions were prepared as described for antisera to A5A (2, 3), except that the anti-S117 sera were adsorbed on Sepharose 2B-coupled IgA/k myeloma proteins TEPC15 and J539. Details on the S117 idiotypic system will be published elsewhere.[2] The Id-binding capacity (IBC) of anti-Id sera and of IgG fractions purified from them was determined at 10^{-8} M radioiodinated Id as previously described (4).

Immunization. Priming was done by intraperitoneal injection of 1×10^9 Strep.A organisms or 0.1 μg IBC of anti-A5A Id1, which was found as effective as the intravenous injections used previously (1). Except for a few experiments in which two priming injections were given at a 2 wk

interval, a single injection was found satisfactory for both Strep.A and anti-A5A Id1. Priming with anti-S117 Id1 was done as with anti-A5A Id1. To induce SRBC priming, 10^8 erythrocytes were injected intraperitoneally. Mice immunized with anti-A5A Id1, anti-S117 Id1, or Strep.A were rested at least 4 wk before being used in experiments. Mice immunized with SRBC were used 9 days later for culture experiments.

Cell Preparations. Mice were killed by cervical dislocation and their spleens were removed sterily and immersed in cold sterile balanced salt solution (BSS). The spleens were then disrupted by teasing and the cell suspensions were allowed to sediment on ice. After 10 min, supernates were separated from sedimented cell debris, centrifuged, and the cell pellets were resuspended and washed in BSS.

Enriched T-cell preparations (85–95% Thy 1.2 positive) were obtained by passage of spleen cells through nylon wool columns according to the method of Julius et al. (17). Spleen cells were depleted of T lymphocytes by treatment with an AKR anti-CBA anti-Thy 1.2 serum and agarose-absorbed (18) guinea pig complement. The characteristics of the anti-Thy 1.2 serum and the methods for T-cell depletion have been described (19). The viability of treated cells was examined by trypan blue exclusion to determine the efficency of specific killing. Then the cells were centrifuged and washed three times in BSS before use in culture experiments.

Lymphocyte Microculture. Lymphocyte microcultures were performed in Micro-Test II tissue culture trays (no. 3040; Falcon Plastics, Div. of BioQuest, Oxnard, Calif.) using a modification of the Mishell and Dutton technique (20). The culture medium, nutritional cocktail, and gas mixture were the same as used in the standard macroculture system (20). Individual wells of a Micro-Test II tissue culture tray were seeded as follows: Antigen was added in 50 μl of medium, followed by 50 μl of medium containing 10^6 spleen cells. For inhibition experiments the 50 μl of cells were added first and subsequently the inhibitor was added in 10 μl of medium. The mixtures were incubated at room temperature for 20 min. Then antigen, in 50 μl of medium, was added.

Inhibition experiments employed purified A-CHO and C-CHO, as well as anti-idiotypic antisera and normal guinea pig serum (NGPS) as control. All guinea pig sera were absorbed twice with 10% (vol/vol) normal spleen cells for 30 min at 4°C and centrifuged for 30 min at 17,000 *g* before use. All inhibitors were present for the entire culture period.

Cultures were maintained without rocking at 37°C in a humid atmosphere of 10% CO_2, 8% O_2, and 82% N_2, and were supplemented on each day with 10 μl of nutritional cocktail. After 4 days the Micro-Test II trays were centrifuged at 100 *g* for 10 min. The culture medium was removed by suction and replaced with 100 μl of BSS. Each culture well was then assayed for direct and indirect plaque-forming cells (PFC) using the local hemolysis in gel assay.

Local Hemolysis in Gel Assay. Antibody-secreting cells were detected by the plaque assay of Jerne et al. (21) as modified by Mishell and Dutton (20), using either A-CHO-SRBC or TNP-SRBC, or both. Attempts were made to detect IgG PFC according to the method of Dresser and Wortis (22) using as developing serum a rabbit antimouse Ig serum of proven ability. There were no anti-TNP IgG-secreting PFC detected in our cultures and we also consistently failed to detect indirect PFC specific for A-CHO even though such cells would have been expected to be generated in culture. This observation agrees with findings of other workers (23, 24). Thus, only the number of direct PFC are presented in this paper. The specificity of PFC was ascertained by plaquing against uncoupled SRBC or by addition of various inhibitors including TNP-bovine serum albumin, A-CHO, or anti-Id, to the agarose gel.

Results

Precursor and Helper Activity In Vitro of Cells Primed In Vivo with Strep.A or with Anti-Id. The experiments were designed in such a way that priming of both B and T lymphocytes in vivo could be detected by challenge of spleen cells with the appropriate antigen in vitro. Priming of B precursor cells was detected by challenge with Strep.A and plaquing against A-CHO-SRBC. Priming of T-helper cells was detected by challenge with TNP-Strep.A and plaquing against TNP-SRBC.

Fig. 1 shows the results of experiments performed to assess the optimum dose

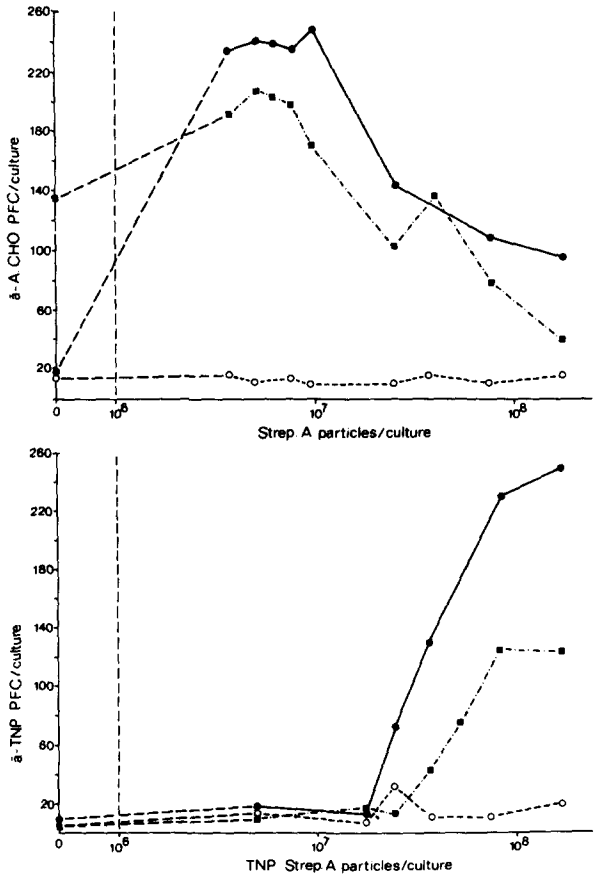

FIG. 1. In vitro PFC response to A-CHO (upper frame), to TNP (lower frame) of normal A/J spleen cells (○---○), and of spleen cells from A/J mice that had been primed with Strep.A (■---■) or anti-A5A Id1 (●——●). Cells were challenged with increasing concentrations of Strep.A particles (upper frame) or Strep.A particles coupled with TNP (lower frame).

for the in vitro challenge with Strep.A (upper frame) and TNP-Strep.A (lower frame), respectively. Spleen cells from normal A/J mice and from A/J mice that had received two intraperitoneal injections of 1×10^9 Strep.A particles or two intraperitoneal injections of 0.1 μg IBC of anti-A5A Id1, respectively, were analyzed in these experiments. As can be seen from the dose-response curves in Fig. 1, cells from mice primed with Strep.A and with anti-A5A Id1 produced substantial numbers of PFC to A-CHO as well as to TNP when challenged in vitro with the appropriate antigen. Spleen cells from unprimed mice failed to respond to either antigen at any of the antigen concentrations tested. The data clearly show that priming with anti-A5A Id1 generates precursor cells for a PFC response to A-CHO as well as helper activity for the response to a hapten that is coupled to Strep.A. Priming with anti-Id is at least as effective as priming with Strep.A. It is apparent that the optimal particle concentration for the induction of anti-TNP PFC is an order of magnitude greater than that for the induction of anti-A-CHO PFC. Similarly, cultures stimulated with TNP-Strep.A for an

optimal anti-TNP PFC response produced hardly any anti-A-CHO PFC (data not shown). Therefore, precursor and helper activity were analyzed in separate experiments throughout this study.

In order to verify that helper function was indeed exerted by T lymphocytes, normal, Strep. A-, or anti-A5A Id1-primed spleen cells were treated either with normal mouse serum and complement or with anti-Thy-1.2 serum and complement before being exposed to TNP-Strep.A in culture. The results from a representative experiment are presented in Table I. Treatment with anti-Thy-1.2 serum and complement completely abolished the ability of all spleen cell preparations to generate TNP-specific PFC on challenge with TNP-Strep.A. Normal mouse serum and complement had little effect on this response. A more direct demonstration that T lymphocytes acted as helper cells in our culture system came from experiments in which nylon wool-enriched T lymphocytes were used. In order to substitute for B cells, untreated spleen cells or anti-Thy-1.2 and complement-treated spleen cells from normal mice were added to the cultures. Control cultures contained normal spleen cells, unfractionated primed spleen cells, or nylon wool-enriched T cells alone. A typical result is presented in Table II. It is evident that unfractionated primed spleen cells and nylon wool-enriched primed spleen cells were equally effective as helpers in the response to TNP-Strep.A. Pretreatment of normal spleen cells with anti-Thy-1.2 serum and complement before mixing with nylon wool-enriched T cells did not reduce the responsiveness of mixed populations to challenge with TNP-Strep.A. Taken together the experiments presented in Tables I and II show that the in vitro anti-TNP PFC response to conjugates of TNP-Strep.A is dependent on the presence of helper T cells primed to Strep.A. The results of this in vitro analysis are essentially in accord with that previously obtained from in vivo adoptive transfers (1), in showing that the helper activity of spleen cells from mice primed

TABLE I

The T-Cell Dependency of the In Vitro Anti-TNP PFC Responses Generated by Strep.A and Anti-A5A Id1-Primed A/J Spleen Cells as Shown by T-Cell Depletion

Priming antigen	Pretreatment of cells	Anti-TNP PFC/culture*
–	–	3 (1.34)
–	NMS + C'‡	3 (1.52)
–	Anti-Thy 1.2 + C'	4 (1.23)
Strep.A	–	175 (1.10)
Strep.A	NMS + C'	108 (1.14)
Strep.A	Anti-Thy 1.2 + C'	7 (1.66)
Anti-A5A Id1	–	152 (1.23)
Anti-A5A Id1	NMS + C'	123 (1.10)
Anti-A5A Id1	Anti-Thy 1.2 + C'	5 (1.28)

* Geometric mean of direct PFC per culture (with standard deviation coefficient). Each result is derived from observations on eight microcultures and is corrected for background anti-SRBC PFC. The dose of TNP-Strep.A in this experiment and in all of the following experiments was 1.5×10^8 particles per culture.

‡ NMS + C', normal mouse serum plus guinea pig complement.

TABLE II

The T-Cell Dependency of the In Vitro Anti-TNP PFC Responses Generated by Strep.A and Anti-A5A Id1-Primed A/J Spleen Cells as Shown by T-Cell Enrichment

Cells in culture		Anti-TNP PFC/culture§
Primed*	Unprimed‡	
–	10^6	17 (1.37)
Strep.A, 10^6	–	282 (1.08)
Strep.A, 5×10^5	10^6	203 (1.11)
Strep.A; T cells, 5×10^5‖	10^6	192 (1.14)
Strep.A; T cells, 1.5×10^6	–	4 (1.70)
–	10^6	82 (1.12)
Anti-A5A Id1, 10^6	–	550 (1.23)
Anti-A5A Id1, 5×10^5	10^6	532 (1.17)
Anti-A5A Id1; T cells, 3.2×10^5	10^6	397 (1.15)
Anti-A5A Id1; T cells 10^6	–	1 (1.11)
–	Anti-Thy 1.2 + C′, 10^6	14 (1.26)
Anti-A5A Id1; T cells, 4×10^5	Anti-Thy 1.2 + C′, 6×10^5	410 (1.07)

* Spleen cells from A/J mice immunized with Strep.A or anti-A5A Id1 as indicated.

‡ Spleen cells from normal A/J mice.

§ See footnotes Table I.

‖ T-cell-enriched population obtained by passage of spleen cells over a nylon wool column.

with anti-A5A Id1 is exerted by T lymphocytes specific for streptococcal antigens.

Specificity and Heterogeneity of Primed Cell Populations. In previous experiments it had been shown that A/J spleen cells primed with anti-A5A Id1 exclusively produced A5A-Id-positive antibodies when transferred into an adoptive host and challenged with Strep.A (1). This is in contrast to cells primed with Strep.A, which produced antibodies only about 25% of which were A5A-Id positive. This result indicated that anti-A5A Id1 specifically activated precursor B cells expressing the A5A Id. In addition to providing data on B cells, the present in vitro system enabled us to assess and compare the specificities of both B- and T-lymphocyte populations.

For the study of B cells, Strep.A- and anti-A5A Id1-primed cells were challenged in vitro with Strep.A and analyzed for A-CHO-specific PFC. Each set of cultured spleen cells was divided into four portions; one was plaqued normally against the A-CHO-SRBC, the second was plaqued in the presence of 20 μg A-CHO, the third was plaqued in the presence of 0.5 μg IBC of anti-A5A serum, and the last was plaqued in the presence of an equivalent dilution of NGPS. Results from a representative experiment are presented in Table III. Addition of A-CHO inhibited the PFC of both Strep.A- and anti-A5A Id1-primed cells by about 80%. NGPS at a final dilution of 1:250 had little effect on the appearance of A-CHO-specific PFC. In contrast, anti-A5A serum at the same dilution reduced the number of PFC generated by Strep.A-primed spleen cells by approximately 40% and the number of PFC generated by anti-A5A Id1-primed cells by 94%. In other experiments Strep.A-primed spleen cells produced as few as 10% A5A-

TABLE III

The Idiotypic Specificity of Precursor Cell Responses to A-CHO Generated by Strep.A and Anti-A5A Id1-Primed A/J Spleen Cells In Vitro, as Shown by Inhibition of Plaque Formation

Priming antigen	PFC-inhibitor*	Anti-A-CHO PFC/culture‡
Strep.A	–	169 (1.04)
Strep.A	20 μg A/CHO	34 (1.11)
Strep.A	1:250 NGPS	120 (1.33)
Strep.A	0.5 μg IBC anti-A5A	100 (1.08)
Anti-A5A Id1	–	249 (1.08)
Anti-A5A Id1	20 μg A-CHO	57 (1.21)
Anti-A5A Id1	1:250 NGPS	204 (1.08)
Anti-A5A Id1	0.5 μg IBC anti-A5A	6 (1.00)

* Inhibitors were added to the agarose solution upon performing the plaque test, not to the culture medium. Figures indicate total amounts per 0.5 ml agarose.

‡ Geometric mean of direct PFC per culture (with standard deviation coefficient). Each result is derived from observations on eight cultures and is corrected for background anti-SRBC PFC.

positive PFC, whereas anti-A5A Id1-primed cells invariably generated more than 90% A5A-positive PFC. It thus seems that immunization with anti-A5A Id1 can drastically expand the A5A-positive PFC precursor pool. These data are in strict accordance with that obtained in vivo (1).

To study the specificity and heterogeneity of helper T cells, Strep.A- and anti-A5A Id1-primed spleen cells were challenged in vitro with TNP-Strep.A and analyzed for TNP-specific PFC. Inhibition experiments analogous to those performed to study B-cell specificity were done, but now the inhibitors were included into the culture medium in order to inhibit helper activity. Cultures in which inhibitions were performed were inoculated with: 0.1 or 0.01 μg of A-CHO or of non-cross-reactive C-CHO, or with 0.1, 0.05, or 0.01 μg IBC of anti-A5A serum or with equivalent dilutions of NGPS. The results from a typical experiment are presented in Table IV and are representative of those obtained in several separate trials.

The data show that neither of the primed helper cell populations was inhibited by C-CHO. In contrast, A-CHO, at both concentrations tested, had a strong inhibitory effect on the response of anti-A5A Id1-primed cells and, to a much lesser extent, on Strep.A-primed cells. A similar but more pronounced difference between Strep.A and anti-A5A Id1-primed helper cells was seen upon inhibition with anti-Id. Pronounced inhibition of anti-A5A Id1-primed helper cells is achieved with anti-A5A serum, whereas the effect of this antiserum on Strep.A-primed helper cells was approximately 10 times less. The PFC numbers of cultures that had contained anti-Id have to be compared to that of the cultures with equivalent dilutions of NGPS. As can be seen, NGPS had a slight toxic effect on the cultures, which was, however, nonspecific and could be readily distinguished from the inhibitory effect of anti-Id. Not shown here are the results of control experiments in which the anti-A5A antiserum was tested for inhibition of the anti-TNP PFC response of SRBC-primed cells. No inhibition was found in these experiments, even with 0.1 μg IBC of anti-A5A serum.

TABLE IV

The Idiotypic Specificity of Helper T-Cells Generated in A/J Mice by Immunization with Strep.A or Anti-A5A Id1, as Shown by Inhibition of Carrier Recognition In Vitro

Priming antigen	Inhibitor*	Anti-TNP PFC/culture‡
–	–	5 (1.42)
Strep.A	–	203 (1.11)
Strep.A	0.1 μg C-CHO	312 (1.21)
Strep.A	0.01 μg C-CHO	215 (1.14)
Strep.A	0.1 μg A-CHO	133 (1.22)
Strep.A	0.01 μg A-CHO	231 (1.06)
Strep.A	NGPS 1:250	108 (1.11)
Strep.A	NGPS 1:500	151 (1.03)
Strep.A	NGPS 1:2,500	185 (1.21)
Strep.A	0.1 μg IBC anti-A5A	54 (1.17)
Strep.A	0.05 μg IBC anti-A5A	133 (1.22)
Strep.A	0.01 μg IBC anti-A5A	176 (1.12)
–	–	3 (1.27)
Anti-A5A Id1	–	186 (1.13)
Anti-A5A Id1	0.1 μg C-CHO	215 (1.15)
Anti-A5A Id1	0.01 μg C-CHO	183 (1.09)
Anti-A5A Id1	0.1 μg A-CHO	39 (1.24)
Anti-A5A Id1	0.01 μg A-CHO	89 (1.18)
Anti-A5A Id1	NGPS 1:250	109 (1.12)
Anti-A5A Id1	NGPS 1:500	169 (1.21)
Anti-A5A Id1	NGPS 1:2,500	201 (1.12)
Anti-A5A Id1	0.1 μg IBC anti-A5A	11 (1.34)
Anti-A5A Id1	0.05 μg IBC anti-A5A	31 (1.40)
Anti-A5A Id1	0.01 μg IBC anti-A5A	59 (1.61)

* Purified A-CHO, C-CHO, anti-A5A serum, or NGPS added to the cultures at the concentration indicated. All figures given represent the final concentration of each inhibitor per culture. The anti-A5A serum used had an IBC of 250 μg/ml.

‡ See footnotes Table I.

These data clearly indicate that anti-A5A Id1-primed helper cells differ from Strep.A-primed helper cells in that the former consist essentially of A5A Id-positive cells, whereas the latter are heterogeneous and consist of a mixture of Id-positive and Id-negative cells. Thus, anti-A5A Id1 expands a specific A5A-positive helper T-cell population, analogous to the expansion of A5A-positive B cells shown above.

Analysis of Helper T Cells Carrying the Id of Myeloma Protein S117. To investigate the question whether the anti-Id antibodies that activate and inhibit T cells are indeed directed against immunoglobulin idiotypes, we used the BALB/c myeloma protein S117, which binds A-CHO and whose Id is expressed on induced antibodies of BALB/c mice immunized with Strep.A. In contrast to antigen-induced antibodies, a myeloma protein is not expected to contain any T-cell-derived receptor molecules, and an anti-Id produced against a purified

myeloma protein can be expected to be exclusively directed to immunoglobulin idiotypes.

Cells from normal BALB/c and from BALB/c mice injected with Strep.A or with anti-S117 Id1 were analyzed in experiments analogous to those performed for the A5A Id in A/J mice. The specificity of the ensuing helper T-cell response was measured by addition of 0.01 μg IBC of anti-S117 serum to cultures containing either of the primed spleen cell populations. Control cultures received an appropriate dilution of NGPS. Cultures were challenged with TNP-Strep.A and analyzed for anti-TNP PFC.

The results in Table V show that spleen cells from unimmunized BALB/c mice failed to generate TNP-specific PFC in culture, whereas spleen cells harvested from Strep.A- or anti-S117 Id1-primed mice produced a substantial TNP-specific PFC response when challenged with TNP-Strep.A. Addition of 0.01 μg IBC of anti-S117 serum to the cultures completely abolished the response of anti-S117 Id1-primed cells, but did not reduce the response of Strep.A-primed cells any more than did NGPS in equivalent dilution. This is in agreement with results on the antibody response of BALB/c mice to Strep.A, which is characterized by a small proportion of S117 Id-positive antibodies (C. Berek and K. Eichmann, unpublished observation). Thus, as in the A5A system of strain A/J, the heterogeneity of A-CHO-binding T-helper cells in BALB/c mice closely reflects the heterogeneity of the corresponding B-cell population. Furthermore, as pointed out above, the anti-Id that react with T-helper cells in the S117 system are unequivocally directed against immunoglobulin idiotypes.

Strain Specificity of T-Cell Idiotypes. The phenotypic polymorphism observed for the A5A and S117 idiotypes in strain A/J and BALB/c antibodies to A-CHO reflects a genetic polymorphism in the genes that encode the V regions of the heavy chains of these antibodies (10). A similar polymorphism on the level of the helper T cells of the two strains would be a further indication for the molecular identity of T- and B-cell receptor V regions. Furthermore, objections such as the contamination of the anti-Id with antigen, or the possible cross-reactivity of the anti-Id with Strep.A could be dismissed.

TABLE V

The Anti-TNP PFC Responses of Normal, Strep.A, and Anti-S117 Id1 Primed BALB/c Spleen Cells Challenged In Vitro with TNP-Strep.A

Priming antigen	Inhibitor	Anti-TNP PFC/culture*
–	–	3 (1.11)
Strep.A	–	145 (1.06)
Strep.A	1:400 NGPS	64 (1.14)
Strep.A	0.01 μg IBC anti-S117‡	57 (1.12)
Anti-S117 Id1	–	97 (1.06)
Anti-S117 Id1	1:400-NGPS	61 (1.12)
Anti-S117 Id1	0.01 μg IBC anti-S117	4 (1.35)

* See footnotes Table I.

‡ The anti-S117 serum used had an IBC of 40 μg/ml.

To analyze idiotypic polymorphism on the helper T-cell level, A/J and BALB/c mice were primed in a crisscross fashion with Strep.A, anti-A5A Id1 or anti-S117 Id1. Their spleen cells were challenged in culture with TNP-Strep.A and 4 days later the number of TNP-specific PFC in each culture was measured. Some cultures of each of the homologously primed spleen cells, i.e. anti-A5A Id1-primed A/J spleen cells and anti-S117 Id1-primed BALB/c spleen cells, received anti-A5A or anti-S117 serum to examine the specificity of priming, and control cultures received NGPS. The results of these experiments are presented in Table VI.

It is apparent from the data that although helper cells were elicited in both strains by priming with Strep.A, only strain A/J produced helper cells in response to priming with anti-A5A Id1 and only strain BALB/c produced helper cells in response to priming with anti-S117 Id1. Furthermore, the response of anti-A5A Id1-primed A/J cells was inhibited by addition of anti-A5A but not anti-S117 serum to the cultures. Conversely, the response of anti-S117 Id1-primed BALB/c spleen cells could be inhibited by addition of anti-S117 but not anti-A5A serum to the cultures. It is clear from the data that helper cell priming as well as inhibition of the helper function by anti-Id reveal the same idiotypic polymorphism as has been observed for the corresponding antibodies.

TABLE VI

The Anti-TNP PFC Responses of A/J and BALB/c Spleen Cells Primed with Strep.A, anti-A5A Id1, or Anti-S117 Id1

Mouse strain	Priming antigen	Inhibitor	Anti-TNP PFC/culture*
A/J	Strep.A	–	179 (1.03)
	Anti-A5A Id1	–	171 (1.08)
	AntiS117 Id1	–	13 (1.29)
	–	–	11 (1.11)
BALB/c	Strep.A	–	94 (1.17)
	Anti-A5A Id1	–	9 (1.74)
	Anti-S117 Id1	–	84 (1.19)
	–	–	7 (1.49)
A/J	Anti-A5A Id1	–	133 (1.16)
	Anti-A5A Id1	0.01 μg IBC anti-A5A‡	28 (1.10)
	Anti-A5A Id1	1:2,500 NGPS	142 (1.15)
	Anti-A5A Id1	0.01 μg IBC anti-S117	83 (1.32)
	Anti-A5A Id1	1:400 NGPS	79 (1.41)
BALB/c	Anti-S117 Id1	–	103 (1.21)
	Anti-S117 Id1	0.01 μg IBC anti-A5A	147 (1.51)
	Anti-S117 Id1	1:2,500 NGPS	162 (1.16)
	Anti-S117 Id1	0.01 μg IBC anti-S117	9 (1.21)
	Anti-S117 Id1	1:400 NGPS	69 (1.31)

* See footnotes Table I.

‡ The anti-A5A serum had an IBC of 250 μg/ml and the anti-S117 serum used had an IBC of 40 μg/ml.

Discussion

The present data confirm our previous observations (1) in showing that anti-Id can presensitize an animal for a secondary response to the antigen which is complementary to the corresponding Id. B cells primed with anti-Id to an A-CHO-binding immunoglobulin generated a strong anti-A-CHO PFC response when challenged with Strep.A in vitro. T cells primed in the same way were able to act as helper cells in the primary immune response to TNP upon challenge in vitro with TNP-Strep.A. Since the magnitude of the responses of cells primed with anti-Id was equal to and, on occasions, exceeded that of cells primed with Strep.A, it appears that priming with anti-Id is as effective as priming with antigen.

The present data extend the previous observations (1), mainly in two ways: (*a*) The in vitro system afforded a detailed analysis of the helper T-cell population that has not been possible in vivo, and (*b*) using the S117 idiotypic system as a counterpart to the A5A idiotypic system it was possible to rule out a number of trivial explanations for the stimulation of lymphocytes with anti-Id. These explanations included the suggestion that our anti-Id preparations were contaminated with antigen, that there exists a cross-reaction between idiotypic determinants of the anti-Id and some streptococcal antigen, and that our anti-Id antisera may contain antibodies to T-cell receptor molecules that may have been copurified with the original A5A antibody and, accordingly, were injected into the guinea pigs together with it. Whereas the former had been considered as rather remote possibilities (1), the latter was taken as a true alternative to account for T-cell activation by anti-Id. However, the experiments that employed the Id of myeloma protein S117 reveal that the anti-Id that interact with T cells are indeed directed against immunoglobulin idiotypes. Since an immunoglobulin secreted by a plasmacytoma tumor is not expected to contain any T-cell-derived receptor molecules, the possibility of anti-T-cell receptor antibodies contaminating the anti-Id antisera is ruled out at least for the S117 system. Furthermore, the strain specificity of lymphocyte sensitization by anti-Id completely eliminates the possibility of antigen contamination as well as the possible cross-reaction between the anti-Id and some streptococcal antigen.

The mechanism by which anti-Id sensitizes B and T lymphocytes is at this stage a matter of speculation. The association of the stimulatory capacity with the IgG1 fraction of anti-Id has been confirmed in unpublished experiments in which IgG2 fractions, isolated from the same sera, were found nonstimulatory at any of the doses (10 ng to 1 μg IBC) at which stimulation was achieved with the IgG1 fractions. However, IgG1 fractions were found to contain small amounts ($<1\%$ of the total IBC) of IgM anti-Id, so that the stimulatory component may not be the IgG1 anti-Id itself. Thus, the requirements for lymphocyte stimulation by anti-Id are poorly understood. No doubt, however, exists that at some stage this process involves the binding of anti-Id to the antigen receptors on the lymphocyte surface. For the helper T cells, this binding is also demonstrated by the inhibition of helper function by anti-Id in vitro. The functional consequences of the binding of anti-Id to antigen receptors imply a functional role in addition to the mere physical presence of Id-bearing molecules on lymphocytes, particularly on helper T cells.

Whereas in the previously reported experiments (1) the specificity and heterogeneity of primed B cells could be studied by idiotypic analysis of antibody products, it was impossible to investigate equivalent properties of the primed helper cell population in vivo. This we have now done in vitro using classical carrier inhibition analysis to ascertain the specificity of T cells primed with antigen and with anti-Id. The results of these experiments clearly revealed that T-helper cells primed with Strep.A were heterogeneous in that only a fraction were inhibitable by A-CHO or anti-Id. This heterogeneity of Strep.A-primed helper cells is probably due to antigenic heterogeneity of the heat-killed and pepsin-treated streptococcus (14) which, in addition to the group-specific carbohydrate antigen, carries peptidoglycan and probably also residual proteins on its surface (25). As immunization with Strep.A gives rise to antibodies against all of these antigens, it is not surprising that a similar spectrum of helper cell specificities is generated, and the partial inhibition of Strep.A-primed helper cells by A-CHO supports this view. The question whether there exists idiotypic heterogeneity within the A-CHO-specific portion of Strep.A-primed helper cells could not be answered beyond doubt, although the results of some experiments appeared to suggest that not all A-CHO-specific helper cells were inhibited by anti-Id. An idiotypic heterogeneity among helper T cells specific for one antigen would indicate the clonal distribution of idiotypes on T cells.

In contrast, helper cells primed with anti-Id were readily and equally well inhibited by anti-Id and A-CHO. This suggests that, out of a heterogeneous population of T-helper cells, anti-Id can identify and expand an apparently uniform cell population. The same was found to be the case for B cells, as shown in the previous paper (1) and confirmed by the present experiments (see Table III). The crucial question to be raised in this connection is whether the process of selecting a specific T-cell population resembles clonal selection as it occurs on the B-cell level. Although this is suggested by the existence of idiotypically uniform and monospecific T-cell populations, a definite answer to this question requires the demonstration that idiotypes are indeed clonal markers not only for B cells but also for T cells.

Another surprising finding which came from these studies is that T cells may have specificity for carbohydrate antigens. The experience of many laboratories that the immune responses to soluble polysaccharide antigens are T-cell independent may have led to the conclusion that T cells are unable to recognize polysaccharide determinants in general. This generalization has been disproved by our experiments. Moreover, T cells can distinguish between carbohydrate antigens as closely related as A-CHO and C-CHO, which are distinct by terminal hexosamines but possess the same rhamnose backbone (25, 26). Thus, the discriminatory capacity of T cells with respect to these antigens is just as good as that of antibodies. This is in agreement with previous studies from this and other laboratories on helper cell specificity (27–29).

The genetic analysis of T- and B-cell idiotypes will be discussed in detail in the accompanying paper (11). In this context it may suffice to mention that both the A5A and S117 idiotypes have been assigned to the V region of the heavy chain of anti-A-CHO antibodies by means of their genetic linkage to the heavy-chain allotype (5, 6, 10). The parallel expression of the same idiotypic polymorphism in

both the B- and T-cell populations suggests that B- and T-cell idiotypes are governed by the same V_H genes, and that the V region of the immunoglobulin H chain is shared between B- and T-cell antigen receptors. Our experiments have no bearing on any other part of the immunoglobulin molecule.

In addition to the sharing of idiotypes between B and T cells, the similarities in specificity and heterogeneity discussed above are further strong arguments in favor of the view that helper T cells use at least partially the same antigen recognition system as B cells, namely the products of H-chain V-region genes. Such conclusions are in agreement with recent experiments of Binz et al., which shown that antibody directed against a specific T-cell alloantigen recognition site also reacts with humoral alloantibody as well as with cells mediating graft vs. host reaction (30, 31). Similarly, McKearn has shown that antialloantibody in rats inhibits graft rejection (32). Binz and Wigzell, however, have also obtained evidence that Id-bearing molecules produced by T cells have properties that clearly distinguish them from immunoglobulin molecules (33). Thus, it appears that evidence for the sharing of idiotypes between T-cell receptors and antibodies has now been obtained in several unrelated experimental systems. Future studies will be directed towards the isolation and characterization of Id-bearing T-cell receptor molecules, using anti-Id as a probe.

Summary

Guinea pig anti-idiotypic antibodies (anti-Id) of the IgG1 class, directed to an A/J antibody to Group A streptococcal carbohydrate (A-CHO), or directed to a BALB/c myeloma protein that binds the same antigen, stimulate B-precursor cells as well as T-helper cells when injected into mice of the appropriate strain. The strain-specific induction of both precursor and helper activity was detected by in vitro secondary responses of primed spleen cells to A-CHO or to 2,4,6-trinitrophenyl (TNP) upon challenge with Group A streptococcal vaccine (Strep.A) or with TNP-Strep.A, respectively. B- and T-cell populations primed with anti-Id were uniform with respect to the binding of antigen and of anti-Id. This was in contrast to cells primed with Strep.A, which were heterogeneous. Taken together, B and T cells that possess the same antigen-binding specificity share idiotypic determinants, reveal the same idiotypic polymorphism, and may display similar degrees of heterogeneity with respect to the binding of antigen and anti-Id. Since the anti-Id used in this study detect Id determinants associated with the heavy chain of the variable region of mouse antibodies, the data suggest that this region of the immunoglobulin molecule is shared between T- and B-cell antigen receptors.

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