EXPRESSION OF Ia ANTIGENS ON HAPTEN-SPECIFIC B CELLS*

I. Delineation of B-Cell Subpopulations

BY JOAN L. PRESS,‡ NORMAN R. KLINMAN, AND HUGH O. McDEVITT

(From the Division of Immunology, Department of Medicine, Stanford University School of Medicine, Stanford, California 94305, and the Department of Pathology, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19174)

The genetic regulation of the murine immune response to a variety of antigens has been found to map in the *I* region within *H-2*, the mouse major histocompatibility complex (1, 2). It is now clear that more than one immune response gene (*Ir* gene) in the *I* region may be involved in the regulation of the immune response to some of these antigens (3, 4). In addition to the complexity of *Ir* gene interaction, there is no definite evidence indicating which cell type(s) express *Ir* gene functions. It is not at all clear whether some or all *Ir* genes are expressed in bone marrow-derived antibody-forming cell precursors (B cells) and/or in thymus-dependent lymphocytes (T cells) required for carrier recognition and helper cell function in the humoral immune response (1, 5–7). Further, it would appear that *I*-subregion identity between B and T cells may be required for effective B-cell–T-cell collaboration (8–10) and/or induction to IgG antibody synthesis (11), even for antigens that are apparently not under *Ir* gene control.

The *I* region also determines the structure of a series of polymorphic cell surface molecules designated Ia, for *I*-region-associated antigens (2). Ia antigens have been demonstrated primarily on lymphocytes, macrophages, sperm, epidermal cells, and late gestation fetal liver cells (12–14). These antigens are easily detected on B cells (12, 14), and the rate of development of Ia-positive cells in the spleen during neonatal ontogeny is closely correlated with that of immunoglobulin (Ig)-bearing cells (15, 16). Several laboratories have been able to detect Ia antigens on thymocytes and T-cell subpopulations as well (17–19), although with more difficulty. Since *I*-region gene products are the major stimulatory determinants for the generation of mixed lymphocyte reactions, T cells presumably recognize B cell (and T cell) Ia antigens (20–22). In light of the evidence that *I*-region homologies facilitate cellular interactions, it has been speculated that Ia antigens may act as regulatory molecules in the immune response, or as recognition sites or signals for B-cell–T-cell interaction and induction (23–25). Ia determinants may also be associated with enhancing (6, 26, 27) and suppressing (28) factors putatively elaborated by activated T cells. If *I*-region gene products are expressed on T cells and these products recognize

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antigen, then these determinants may function as antigen recognition units (29).

The studies reported in this communication were initiated *(a)* to determine whether Ia antigens are expressed on all hapten-specific primary and secondary B cells; *(b)* to determine whether the expression of Ia antigens can be used as a marker to delineate primary B-cell subpopulations; and *(c)* to determine whether Ia antigens play a role in the antigenic stimulation of B cells, particularly with regard to T-cell–B-cell interactions. Spleen cells from nonimmune (primary) and previously immunized (secondary) donor mice were negatively selected by pretreatment with an anti-Ia antiserum and complement and then analyzed for precursor cell activity by an in vitro splenic focus technique for B-cell cloning (30) which maximizes carrier help for B-cell stimulation. The results of these experiments indicate that *(a)* the majority of primary precursor cells and almost every secondary precursor cell is Ia positive; *(b)* a finite subpopulation of Ia-"negative" primary precursor cells exists in the spleens of nonimmune mice; and *(c)* the Ia-negative primary B-cell subpopulation contains those precursor cells which give rise solely to IgM antibody production, whereas the Ia-positive B-cell subpopulation contains precursor cells capable of giving rise to IgG antibody production, or both IgG as well as IgM antibody, under appropriate stimulatory conditions. It has been demonstrated recently that a significant proportion of primary precursor cells can be stimulated in the carrier-primed allogeneic environment, but that only IgM antibody production is elicited. This effect has been mapped to the *I-A-I-B* subregions (11). Since the studies reported here demonstrate that the majority of primary precursor cells are Ia positive and have the capacity to give rise to IgG antibody production upon syngeneic T-cell interaction, these results suggest that Ia antigens may play a role in, or correlate with, the ability of the B cell to switch from IgM to IgG antibody production. The results also suggest that Ia recognitive interactions between T cells and B cells are not required for stimulation to IgM antibody production, even when B-cell stimulation is T-cell dependent.

Materials and Methods

Animals and Immunization. Breeding pairs of the congenic line BALB.K (*H-2^k*) were provided by Dr. Frank Lilly. This congenic line was maintained by brother-sister mating. Mice were carrier primed with 0.1 mg of *Limulus polyphemus* hemocyanin (Hy)[1] in complete Freund's adjuvant (CFA) 2–3 mo before use as recipients for cell transfer. Donor spleen cells were obtained either from nonimmune mice (primary precursor cell analysis) or from mice immunized 2–4 mo previously with 0.1 mg of 2,4-dinitrophenylated Hy (DNP-Hy; 10 mol of DNP per 100,000 g of Hy) (31) in CFA (secondary precursor cell analysis).

Irradiation. Carrier-primed (Hy) recipient mice were lethally irradiated 6 h before cell transfer with 1,100–1,300 R from a Phillips X-ray generator, at 15 mA and 250 kV, with a corrected 0.25 mm Cu/1.0 mm Al filter, half value layer (HVL)-1.3 mm Cu, delivering 62 R/min, at a distance of 64 cm to midmouse.

Antiserum and Complement. A.TH anti-A.TL antiserum (anti-Ia^k), A.TL anti-A.TH antiserum (anti-Ia^s), and (A.TH × B10.HTT)F$_1$ anti-A.TL antiserum (anti-*IA^k*, *IB^k*) were produced in this laboratory by methods described previously (12, 13). These sera were routinely analyzed for

[1] *Abbreviations used in this paper:* CFA, complete Freund's adjuvant; DNP, 2,4-dinitrophenyl; FCS, fetal calf serum; Hy, hemocyanin; MEM, minimal essential medium; NMS, normal mouse serum; PBS, phosphate-buffered saline.

specificity and cytotoxic activity by a complement-mediated microcytotoxicity assay employing trypan blue dye exclusion (12). Using the appropriate target strain and antiserum combinations, these anti-Ia antisera cytolyze 50–60% of splenic lymphocytes with a cytotoxic titer >1/1,000. Most of the experiments utilized BALB.K spleen cells and A.TH anti-A.TL antisera or A.TH normal mouse serum (NMS). (B10 × HTI)F$_1$ anti-B10.A(5R) was the kind gift of Dr. Donal Murphy and had cytotoxic antibody activity against products of the IC^d subregion, which shares cross-reacting Ia specificities with IC^k (32).

Fresh frozen rabbit serum was used as the complement (C′) source. Rabbit serum was adsorbed with agarose, and the complement activity and nonspecific toxicity screened before use by methods described previously (12, 13).

Pretreatment of Donor Spleen Cells. Spleens from adult, nonimmune, or DNP-Hy immunized mice were exhaustively flushed with Dulbecco's phosphate-buffered saline (PBS, Grand Island Biological Co., Grand Island, N. Y.) supplemented with 5% heat-inactivated fetal calf serum (FCS). Cell viability, measured by trypan blue dye exclusion, was usually >90%.

100 × 10^6 spleen cells were pelleted in plastic Falcon tubes (Falcon Plastics, Oxnard, Calif.), resuspended in 1 ml of NMS or anti-Ia antiserum diluted $^1/_5$ in PBS-FCS, and incubated for 25 min at 4°C. After washing in PBS-FCS, the pretreated spleen cells were then incubated in 3–4 ml of adsorbed rabbit complement, diluted $^1/_8$–$^1/_{12}$ in Dulbecco's minimal essential medium (MEM) (GIBCO) for 45 min in a 37°C, 95% air-5% CO_2 aerated incubator. After C′ incubation, the cell suspensions were immediately placed on ice, and the percent cytotoxicity was measured by dye exclusion. The cells were washed three times in MEM and adjusted to a cell concentration of 1–2 × 10^7 viable cells/ml. Lethally irradiated, carrier-primed recipients received 1–2 × 10^6 viable, pretreated cells intravenously (i.v.). The number of total donor cells injected was calculated on the basis of the final cell count measured by dye exclusion before cell injection.

Fragment Cell Culture and Radioimmunoassay. The frequency of hapten-specific clonal precursor cells was measured by an in vitro splenic focus technique for B-cell cloning. The methodology of this technique and the detection of positive (anti-hapten antibody producing) fragment cultures have been described previously (30). 14–16 h after cell transfer, recipient spleens were sliced into 1-mm cubes, placed in vitro in organ fragment culture, and stimulated for 3 days with 1 × 10^{-6} M DNP-Hy. Culture fluids were changed and collected at 2- to 3-day intervals thereafter.

The production of anti-DNP monofocal antibody by the splenic foci was determined by a sensitive, relatively affinity-independent radioimmunoassay using a DNP-lysine-conjugated-bromoacetyl cellulose immunoadsorbent and ^{125}I-labeled purified rabbit anti-mouse Fab antibody (30, 31). The heavy chain class of anti-DNP monofocal antibody produced by each clone was also determined by the radioimmunoassay using iodinated, purified goat antibodies against either the mouse μ- or γ_1-heavy chain class (30, 33).

Results

The frequency of DNP-specific precursor cells in the spleens of nonimmune and DNP-Hy immunized adult BALB.K mice was quantitated after pretreatment either with A.TH NMS or with A.TH anti-A.TL antiserum (anti-Ia^k) and complement. Limiting numbers (1–2 × 10^6 viable cells per recipient) of pretreated spleen cells were injected into lethally irradiated, carrier (Hy)-primed, syngeneic recipients. No primary foci were detected after transfer of either NMS or anti-Ia^k and complement-pretreated cells into lethally irradiated, normal syngeneic recipients. This is consistent with previous findings that carrier-primed recipients are necessary for the induction of primary B-cell clones by DNP-Hy (30, 34).

After pretreatment with NMS and complement, the average frequency of BALB.K primary precursor cells specific for DNP ranged from 6–10 foci per 10^6 total injected cells, with a mean frequency of 7.64 foci per 10^6 cells (Table I). This frequency is similar to that obtained with untreated, nonimmune spleen cells (data not shown). When the spleen cells were pretreated with anti-Ia^k and

complement, however, the mean precursor cell frequency was lowered to 0.94 foci per 10^6 total injected cells, a reduction of 88%. While the majority of DNP-specific primary B cells are thus Ia positive, a small amount of primary precursor cell activity does remain after negative selection. Pretreatment of nonimmune spleen cells with the reciprocal antiserum, A.TL anti-A.TH (anti-Ia^s), does not reduce the precursor cell frequency (Table II); this is expected, since the $H\text{-}2^k$ and $H\text{-}2^s$ haplotypes do not share cross-reacting Ia specificities when antisera produced by reciprocal immunization are used (2, 14). Table II also demonstrates that this negative selection procedure is complement dependent.

The results of three experiments in which spleen cells from BALB.K mice previously immunized to DNP-Hy in CFA were treated with anti-Ia^k and complement, and subsequently analyzed for precursor cell activity, are presented in Table III. The mean frequency of NMS-treated DNP-specific secondary precursor cells is 13.46 foci per 10^6 injected cells. This frequency was reduced to 0.59 per 10^6 cells after negative selection, a reduction of 96%. Hence, secondary precursor cells can be almost totally eliminated.

The monofocal anti-DNP antibodies produced by the primary and secondary clones presented in Tables I and III were analyzed for their isotype (heavy chain class) by radioimmunoassay. The distribution of primary and secondary clones producing IgM, IgG_1, or both IgM and IgG_1 anti-DNP antibody is shown in Table IV. After pretreatment of the donor spleen cells with NMS and complement, an average of 72% of the clones produced only IgG_1 antibody; 14% produced only IgM antibody; and 14% produced both IgM and IgG_1 antibody (double producers). Less than 10% of the clones detected by ^{125}I-anti-Fab antibody produced anti-DNP antibody which was neither of the μ- or γ_1-heavy chain class. This isotype distribution of anti-DNP monofocal antibodies produced by BALB.K primary clones is similar to that observed for BALB/c clones (33). Primary clones derived from spleen cells pretreated with anti-Ia^k and complement gave an isotype distribution markedly altered from that of the NMS and complement control group. An average of 78% of the clones generated from the remaining primary B cells produced solely IgM anti-DNP antibody; 18% produced IgG_1 antibody; and 4% produced both IgM and IgG_1 antibody. The secondary monofocal antibody isotype distribution is distinct from that of primary monofocal antibodies: an average of 2% of secondary clones produced solely IgM anti-DNP antibody; the other clones produced either IgG_1 or both IgM and IgG_1 antibody. After negative selection, however, the majority of clones derived from the secondary spleen cell population produced solely IgM antibody.

The selective survival of purcursor cells which give rise to solely IgM antibody-producing clones is further illustrated in Fig. 1 and 2. From the data presented in Tables I, III, and IV, the frequency per 10^6 total injected cells of primary and secondary precursor cells giving rise to clones producing antibody of the μ, γ_1, or both μ and γ_1 heavy chain class was calculated. It is apparent that the reduction in the total primary precursor cell frequency is correlated with the marked reduction (>95%) in the mean frequency of primary B cells giving rise to IgG_1 antibody-producing clones (Fig. 1). In contrast, on a total cell basis, the mean frequency of primary precursor cells which give rise to solely IgM antibody-producing clones is only slightly lowered (20–30%). The precursor

TABLE I

Effect of Anti-Ia^k and Complement on the Frequency of DNP-Specific Primary Precursor Cells

Exp.	Pretreatment*		No. viable donor cells/recipient‡	No. total donor cells/recipient	No. clones detected/recipient	Total no. clones	No. total donor cells injected	Average frequency/10^6 total cells§	Reduction in clonal frequency
	Serum	Kill							
		%							%
1	NMS/C′	15	1.0×10^6	1.1×10^6	13,9	22	2.2×10^6	10.0	92
	Ia^k/C′	62	1.0×10^6	2.8×10^6	2,1,4	7	8.4×10^6	0.83	
2	NMS/C′	26	1.0×10^6	1.2×10^6	7,8,6	21	3.6×10^6	5.83	92
	Ia^k/C′	63	1.0×10^6	2.9×10^6	1,0,3	4	8.7×10^6	0.46	
3	NMS/C′	11	1.8×10^6	1.9×10^6	19,11	30	3.8×10^6	7.89	78
	Ia^k/C′	62	2.1×10^6	3.8×10^6	7,6,7	20	11.4×10^6	1.75	
4	NMS/C′	9	2.0×10^6	2.2×10^6	15	15	2.2×10^6	6.82	90
	Ia^k/C′	67	2.0×10^6	3.3×10^6	3,3,1	7	9.9×10^6	0.71	
							Average of four exp:‖		
							NMS/C′	7.64 (0.45)	88
							Ia^k/C′	0.94 (0.14)	

* BALB.K donor spleen cells were incubated with 1 ml of 1/5 A.TH NMS or A.TH anti-A.TL antiserum per 100×10^6 cells at 4°C for 25 min, washed, and then incubated with rabbit complement ($^1/_8 - {}^1/_{12}$) at 37°C for 45 min. Percent cytotoxicity was measured by trypan blue dye exclusion immediately thereafter.

‡ Spleen cells washed three times after complement incubation; cell viability measured by dye exclusion.

§ Average DNP-specific clonal precursor cell frequency calculated by dividing total number of detected clones by total number of cells injected.

‖ Mean frequency per 10^6 total cells; numbers in parentheses indicate standard error of the mean.

TABLE II

Specificity of Precursor Cell Frequency Reduction

Serum pretreatment*	Addition of C′	Mean precursor cell frequency per 10^6 total cells‡
NMS	+	6.97 (0.92)
Ia^s	+	7.87 (1.74)
Ia^k	−	4.69 (1.44)
Ia^k	+	0.80 (0.12)

* BALB.K primary spleen cells were incubated with A.TH NMS, A.TH anti-A.TL antiserum (Ia^k), or A.TL anti-A.TH antiserum (Ia^s) for 25 min at 4°C, washed, and then incubated for 45 min at 37°C in the presence or absence of rabbit complement (C′).

‡ Mean precursor cell frequency of three experiments; numbers in parentheses are the standard error of the mean.

cell contribution to the total primary IgM response is reduced by pretreatment with anti-Ia^k and complement, since the contribution to total IgM antibody by clones producing both IgM and IgG_1 antibody has been depleted (>90%) as well. Pretreatment of secondary spleen cells reduced the frequency of secondary B cells giving rise to IgG_1 antibody-producing clones by 95% and the frequency of secondary B cells giving rise to both IgG_1 and IgM antibody-producing clones by 100% (Fig. 2). The low frequency of precursor cells in the secondary spleen cell population giving rise to only IgM antibody-producing clones was not affected.

Two "restricted" Ia antisera were also analyzed for their ability to reduce the DNP-specific primary precursor cell response. Spleen cells from BALB.K mice were treated with an (A.TH × B10.HTT)F_1 anti-A.TL serum (023) or with a (B10

TABLE III
Effect of Anti-Ia^k and Complement on the Frequency of DNP-Specific Secondary Precursor Cells

Exp.	Pretreatment*		No. viable donor cells/recipient‡	No. total donor cells/recipient	No. clones detected/recipient	Total no. clones	No. total donor cells injected	Average frequency/10^6 total cells§	Reduction in clonal frequency
	Serum	Kill							
		%							%
1	NMS/C′	14	1.0×10^6	1.3×10^6	16,19,13	48	3.9×10^6	12.31	91
	Ia^k/C′	64	1.0×10^6	2.1×10^6	4,1,2	7	6.3×10^6	1.11	
2	NMS/C′	14	1.0×10^6	1.3×10^6	12,18,15	45	3.9×10^6	13.64	96
	Ia^k/C′	59	1.0×10^6	2.2×10^6	2,1,1,0	4	6.6×10^6	0.60	
3	NMS/C′	15	1.0×10^6	1.2×10^6	22,16,14	52	3.6×10^6	14.44	99
	Ia^k/C′	54	1.0×10^6	1.5×10^6	0,0,0,0.5	0.5	6.0×10^6	0.08	
								Average of three exp:‖	
							NMS/C′	13.46 (0.62)	96
							Ia^k/C′	0.59 (0.29)	

* Spleen cells from BALB.K mice primed with 0.1 mg DNP-Hy in CFA 6–8 wk previously were incubated with 1 ml of 1/5 A.TH NMS or A.TH anti-A.TL antiserum (Ia^k) per 100×10^6 cells at 4°C for 25 min, washed, and then incubated with rabbit complement ($^1/_8 - ^1/_{12}$) at 37°C for 45 min. Percent cytotoxicity was measured by trypan blue dye exclusion immediately thereafter.

‡ Spleen cells washed three times after complement incubation; cell viability was measured by dye exclusion. Cells transferred i.v. into Hy carrier-primed, irradiated BALB.K mice.

§ Average DNP-specific clonal precursor cell frequency calculated by dividing total no. of detected clones by total no. of cells injected.

‖ Mean frequency per 10^6 total cells; numbers in parentheses indicate standard error of the mean.

× HTI)F$_1$ anti-B10.A(5R) serum (As120) and complement. The former antiserum has antibody activity against gene products of the *I-A^k* and *I-B^k* subregions, while the latter antiserum reacts with gene products of the *I-C^d* subregion and cross-reacts with products of the *I-C^k* subregion (32). As120 may also contain antibodies reactive with gene products of the *S^d* subregion (D. B. Murphy, personal communication). Treatment with either antiserum significantly reduced primary precursor cell activity (Table V). Similar results were obtained when an A.TH anti-A.TL serum was used with BALB.B (*H-2^b*) or BALB/c (*H-2^d*) spleen cells (data not shown), an expected finding since the *H-2^b* and *H-2^d* haplotypes share some cross-reacting Ia specificities with the *H-2^k* haplotype (2, 14, 29).

Discussion

The studies presented here were conducted to determine whether Ia antigens are expressed on DNP-specific B cells and whether the expression of Ia determinants can be used to delineate B-cell subpopulations in the spleens of nonimmune and previously immunized mice. To measure the relevance of Ia expression to specific precursor cell stimulation on a cell to cell basis, and under conditions of T-cell excess, the experimental approach employed negative selection with specific anti-Ia serum and complement and an in vitro splenic focus technique (30) for B-cell cloning. The splenic focus technique allows the enumeration of clonal precursor cells in the population with and without negative selection and permits an analysis of effects of such selection at the level of the individual B cell and its clonal progeny.

It has been demonstrated using this splenic focus technique that the limiting cell for focus formation is the B cell, and that the monofocal antibody produced is derived from the clonal progeny of a single stimulated precursor cell (30, 34).

TABLE IV

Effect of Anti-Ia^k and Complement on the Isotype of Monofocal Antibody Produced Per Clone

Exp.*	Pretreatment	No. clones producing monofocal antibody of isotype‡				Clones producing monofocal antibody of isotype		
		μ	γ_1	$\mu + \gamma_1$	(Fab)§	μ	γ_1	$\mu + \gamma_1$
							%	
Primary precursor cell								
1	NMS/C′	3	18	1	–	13.6	81.8	4.6
	Ia^k/C′	6	1	0	–	85.7	14.3	0
2	NMS/C′	4	15	2	–	19.0	71.4	9.6
	Ia^k/C′	4	0	0	–	100	0	0
3	NMS/C′	3	20	7	–	10.0	66.7	23.3
	Ia^k/C′	14	3	3	–	70.0	15.0	15.0
4	NMS/C′	2	10	3	–	13.3	66.7	20.0
	Ia^k/C′	4	3	0	–	57.1	42.9	0
Secondary precursor cell								
1	NMS/C′	0	23	25	–	0	47.9	52.1
	Ia^k/C′	4	3	0	–	57.1	42.9	0
2	NMS/C′	0	9	36	–	0	20.0	80.0
	Ia^k/C′	2	2	0	–	50.0	50.0	0
3	NMS/C′	3	26	20	3	5.8	50.0	38.5
	Ia^k/C′	0.5	0	0	0	100	0	0

* See Tables I and III for details.

‡ DNP-specific clones from Tables I and III analyzed for heavy chain class by radioimmunoassay.

§ Fab refers to clones detected by ^{125}I rabbit anti-mouse Fab which are not detected by either iodinated anti-μ or anti-γ_1 antibody; in general, such clones represent <10% of the total clones.

The majority of splenic foci produce antibody that appears homogeneous by the criteria of heavy and light chain recombination analysis, equilibrium dialysis, idiotypic analysis, and isoelectric focusing (30, 34, 35). The only exception to the evidence for the homogeneity of the monofocal antibody produced by these clones has been the demonstration that a significantly greater than random frequency of individual clones produce monospecific anti-DNP antibody in both the μ- and γ_1-heavy chain classes (33). The occurrence of double producers and the implication that the clonal progeny of a single stimulated precursor cell may produce antibody in more than one heavy chain class specific for a single determinant has recently been confirmed by the studies of Gearhart et al. (35), who demonstrated that monospecific, antiphosphorylcholine antibodies from the same clone were of more than one heavy chain class, yet share the same specificity region by idiotypic analysis.

Expression of Ia Antigens on B-Cell Subpopulations. Inasmuch as the secondary precursor cell frequency is almost totally ablated by negative selection,

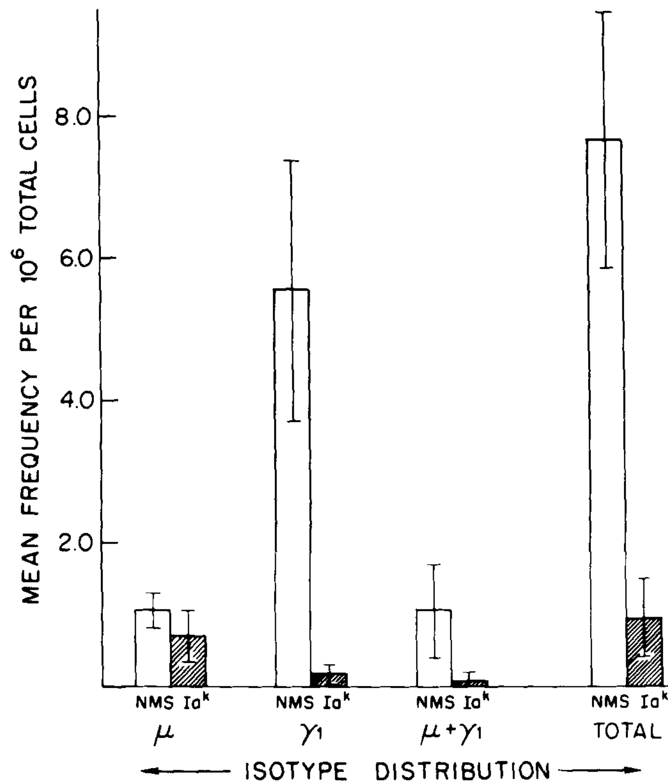

FIG. 1. Primary DNP-specific precursor cell frequency per 10^6 total injected cells after pretreatment with A.TH NMS or A.TH anti-A.TL (Ia^k) serum and complement. Heavy chain class (isotype) of antibody produced per clone determined by a radioimmunoassay. Mean frequency and standard deviation.

almost every secondary B cell must be Ia positive. In contrast, a small but discernible amount of primary B-cell activity is retained after negative selection, suggesting that some primary B cells are Ia negative. The terminology Ia negative includes those B cells which may possess Ia molecules on their surface, but in quantity or density too low to permit cytolysis by antibody and complement, or detection by immunofluorescent analysis on the fluorescence-activated cell sorter (36). The reduction in the primary B-cell frequency is due primarily to the elimination of B cells which would have given rise to clones producing IgG_1 anti-DNP antibody. The frequency of precursor cells generating solely IgM-producing progeny is only slightly affected. However, the overall primary IgM antibody response is affected since the precursor cells which yield clones producing both IgG and IgM antibody are Ia positive and are eliminated as well. It is not clear whether the low frequency of Ia-negative precursor cells and the quantity of IgM antibody elaborated by their clonal progeny would be sufficient to permit detection by either in vivo or other in vitro techniques. It has been possible to demonstrate in an in vivo cell transfer system employing excess helper T cells that the primary IgG antibody response to ferritin is significantly diminished when the nonimmune donor spleen cells are negatively selected with

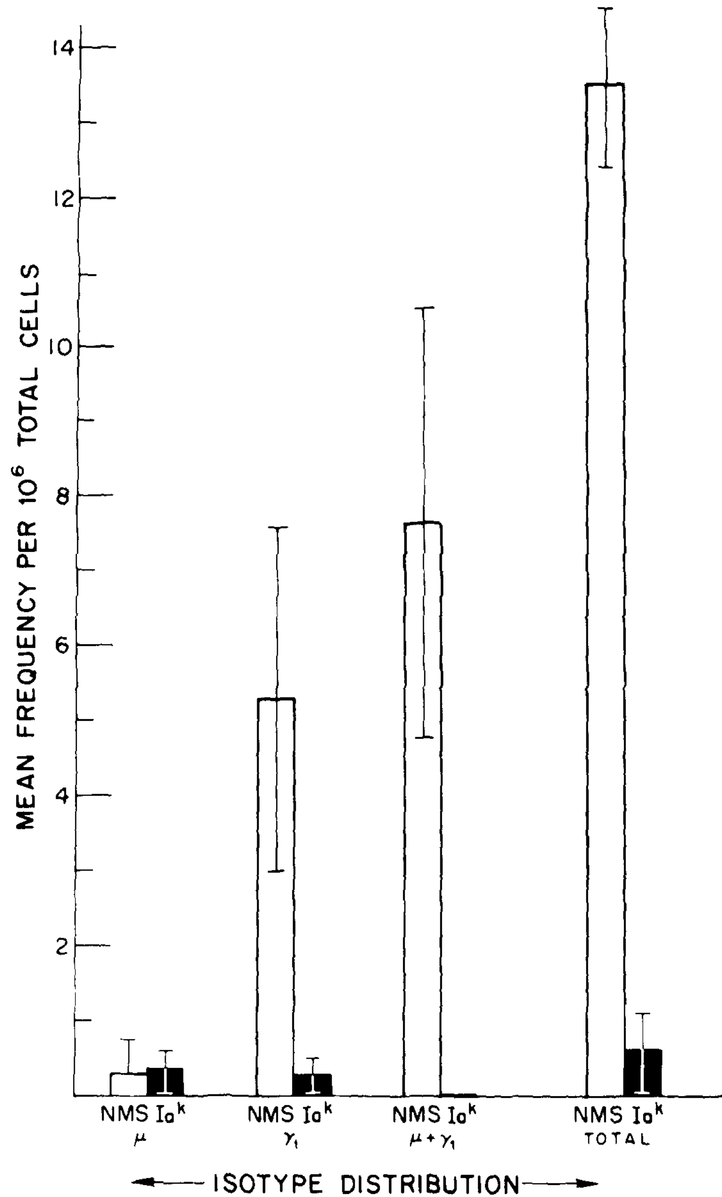

FIG. 2. Secondary DNP-specific precursor cell frequency per 10^6 total injected cells after pretreatment with A.TH NMS or A.TH anti-A.TL (Ia^k) serum and complement. Heavy chain class (isotype) of antibody produced per clone determined by a radioimmunoassay. Mean frequency and standard deviation.

Ia antiserum and complement before cell transfer (J. Press, S. Strober, and H. McDevitt. Manuscript in preparation). The negative selection procedure is complement dependent and apparently Ia determinant specific, since restricted antisera directed against antigens specified by the *I-A* and *I-B*, or *I-C* subregions are also effective in reducing the primary B-cell response.

TABLE V
Expression of I Subregion Defined Ia Specificities on Primary B Cells

Exp.	Serum* pretreatment	Kill	Frequency per 10^6 total cells Isotype distribution				Total reduction	Potential *I* subregions detected
			μ	γ_1	$\mu + \gamma_1$	Total‡		
		%					%	
1	NMS	11	0.21	5.83	0.42	6.46		
	023	57	0.19	1.86	0.09	2.13	67	*I-A*, *I-B*
2§	014	13	2.5	4.2	1.8	9.6		
	023	50	2.1	0.77	0.38	3.5	64	*I-A*, *I-B*
	As120	54	1.5	0.19	0	2.2	77	*I-C*

* Antisera used: NMS, A.TH normal mouse serum; 014, A.TL anti-A.TH (anti-$I\text{-}A^s$, $I\text{-}B^s$, $I\text{-}C^s$, S^s, G^s); 023, (A.TH × B10.HTT)F_1 anti-A.TL (anti-$I\text{-}A^k$, $I\text{-}B^k$); As120, (B10 × HTI)F_1 anti-B10.A(5R) (anti-$I\text{-}C^d$, S^d).

‡ Total clonal precursor cell frequency per 10^6 injected cells, including clones detected with ^{125}I-anti-Fab, but not ^{125}I-anti-μ or anti-γ_1 antibody.

§ In this experiment, 014 is used as the control serum vs. $H\text{-}2^k$.

The expression of Ia antigens thus appears to define two subsets of primary B cells. The majority of primary B cells are Ia positive and have the potential to generate IgG-producing progeny. There is also a small subpopulation of B cells which is by definition Ia negative. These B cells generate only IgM-producing clones under the conditions employed in these studies. Ia-positive and Ia-negative B cells can therefore be demarcated by their clonal phenotype. Two other pieces of evidence indicate that Ia-negative B cells exist as a discrete subset: (*a*) when nonimmune spleen cells are separated by immunofluorescence on the cell sorter, significant precursor cell activity can be detected in the Ia-negative cell population (J. Press, unpublished observations); (*b*) analysis by size fractionation on 1 *g* velocity sedimentation gradients indicates that the Ia-negative B cells which give rise to only IgM-producing clones are found predominantly in the large cell fractions, whereas both the large and small cell fractions contain Ia-positive primary B cells (37).

The interrelationship of the Ia-positive and Ia-negative B-cell subsets is not clear. A continuum may exist within the nonimmune adult spleen, where Ia-negative (and possibly Ig negative) B cells differentiate into Ia-positive cells. In this single pathway model, the B cell would acquire the capacity to switch to IgG production concomitant with the expression of Ia, and the expression of Ia molecules could be used as a marker to follow B-cell maturation. Alternatively, the Ia-positive and negative B-cell subsets may constitute separate, parallel lineages. The Ia-negative B-cell subset would exist in low frequency in the adult spleen and would not diferentiate into Ia-positive cells. Preliminary findings indicate that the IgM-producing clonal progeny of Ia-negative B cells do not express Ia antigens during the in vitro culture period (J. Press and N. Klinman, unpublished observations). It is not clear whether Ia-negative B cells are capable of making IgG antibody but do not due to the lack of Ia determinant expression, or whether such cells are intrinsically capable of producing only IgM

antibody upon stimulation. It will be of interest to determine whether the Ia-positive and Ia-negative B-cell subsets share the same repertoire of antibody clonotypes and express the same requirements for stimulation, particularly with regard to T-cell dependent versus T-cell independent antigens.

Inasmuch as the expression of Ia antigens on the B cell correlates with the ability of that cell to give rise to IgG production, Ia molecules may have a role in the specific induction of the B-cell switch mechanism from IgM to IgG production. Indirect support for this speculation comes from recent findings of Pierce and Klinman (11) that 60–70% of primary B cells can be stimulated in allogeneic, carrier-primed recipients. This stimulation does not occur if the allogeneic recipient is not carrier primed. When the donor and recipient haplotype combinations were selected such that there was genetic nonidentity only in the *I-A* and *I-B* subregions, then antigen-specific stimulation yielded clones producing solely IgM anti-DNP antibody. In contrast, if there were background differences only, or differences in the *H-2D* end, then IgG antibody-producing clones were obtained (11). These findings have been confirmed using several congenic and recombinant strain combinations, but there may be preferred *H-2* haplotype combinations for optimal stimulation (J. Press and N. Klinman, unpublished observations).

Thus, gene products affecting the ability of B-cell–T-cell interactions to lead to IgG production map in the *I* region (7–11). However, Ia recognitive interactions between B cells and T cells do not appear obligatory for B-cell triggering per se, since Ia-negative cells can be specifically stimulated to IgM antibody production. These data suggest that the Ia-positive primary B cell can give rise either to IgM or to IgG production, depending on whether there is *I*-region identity between the B cell and T cell. This is compatible with the hypothesis that Ia antigens are involved in the B-cell induction to IgG production. The requisites for *I*-region identity between B cells and T cells for IgG production may be more stringent for primary B cells than for secondary B cells (38; J. Press, unpublished observations). In situations where IgG antibody is elicited in histoincompatible T-cell–B-cell interactions, e.g. tetraparental mice (5) or bone marrow chimeras (39), this could be due either to tolerance of alloreactive T cells or to the learned recognition of both parental haplotype cell surface *I*-region antigens as a consequence of in situ immunization.

It is clear that the expression of Ia molecules on B cells can be used to classify at least two DNP-specific primary B-cell subpopulations in the spleens of adult mice. At issue is whether Ia expression is a B-cell differentiation event in a common lineage pathway and whether Ia antigens per se have any functional role in the IgM to IgG switch mechanism. The cellular expression of Ia molecules may constitute an inherent molecular mechanism for the regulation of B-cell stimulation. This regulatory role of Ia determinants could be quite distinct from the mechanism of action and antigen specificity of *Ir* genes. Experiments are in progress to resolve these issues.

Summary

The nonimmune adult spleen contains at least two B-cell subpopulations. The majority of primary B cells express cell surface Ia determinants and have the

capacity to give rise to IgG antibody-producing clones after T-cell dependent antigenic stimulation. There is also a small subpopulation of primary B cells which are, by definition, Ia negative, since their activity is not eliminated by negative selection with anti-Ia serum and complement. The Ia-negative B cells give rise to clones that produce only IgM antibody. These B-cell subsets may form a continuum in B-cell maturation, or they may exist as discrete B-cell lineages. Since the cellular expression of Ia antigens appears to correlate with the ability of the B cell to generate IgG-producing clones, it is speculated that Ia molecules may have a role in the IgM to IgG B-cell switch mechanism.

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