SEROLOGIC IDENTIFICATION OF THE HUMAN SECONDARY B CELL ANTIGENS

Correlations between Function, Genetics, and Structure*

By STEPHEN SHAW, ROBERT DeMARS, STUART F. SCHLOSSMAN, PAULA L. SMITH, LOIS A. LAMPSON, AND LEE M. NADLER

From the Immunology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205; the Laboratory of Genetics and Department of Human Oncology, University of Wisconsin, Madison, Wisconsin 53706; the Division of Tumor Immunology, Sidney Farber Cancer Institute, Boston, Massachusetts 02115; and the Department of Anatomy, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104

Ia antigens are a class of cell surface glycoproteins encoded within the murine major histocompatibility complex (MHC)[1] that have a characteristic molecular structure and tissue distribution. The genetics, structure, and function of these molecules are being investigated intensively because Ia antigens have been found to be involved in antigen recognition by T lymphocytes and are believed to regulate antigen recognition by those T lymphocytes (1, 2). In man, HLA-DR is the only Ia-like molecule that has been well characterized with respect to: (*a*) determination of its molecular structure; (*b*) mapping the gene that encodes it; and (*c*) assigning it a role in human T cell recognition (3–5). However, multiple lines of evidence indicate that there is at least one other, and perhaps many more, human Ia-like gene products. This complexity is evident from serologic (6–9) and immunochemical (10–19) analyses; however, little is known about the ability of these determinants to function in T cell recognition (20), and the available genetic data (7, 21) are insufficient to conclusively establish that these determinants are controlled by genes distinct from HLA-DR. Such correlations between structure, genetics, and function will have to be established to achieve a more general understanding of human Ia antigens.

Recently, cellular typing reagents were developed that defined five alleles of a new segregant series of secondary B cell (SB) antigens (22, 23); they were shown to be encoded by an HLA-linked locus that was distinguished from *HLA-DR* by identification of recombinant families (24) and studies of mutant cell lines (25). It was hypothesized on the basis of function and tissue distribution that the SB gene product is an Ia-like molecule (22). Independently, a monoclonal antibody was identified (18, 26) that reacted with an antigen whose molecular structure was similar to HLA-DR, but that could be distinguished from the HLA-DR molecule by sequential immunoprecipitation. One relatively unique characteristic of ILR1 reactivity and of the SB antigens made it plausible that both might relate to the same genetic system—although each was encoded by a locus linked to *HLA-DR*, in neither case did the

* Supported in part by grant AI-15486 from the National Institutes of Health.

[1] *Abbreviations used in this paper:* FMF, flow microfluorometry; LCL, lymphoblastoid cell line; MHC, major histocompatitibility complex; SB, secondary B cell.

polymorphism correlate well with HLA-DR in the population.

Three lines of evidence described in this report indicate that this monoclonal antibody, ILR1, reacts with at least two allelic forms of the SB molecule: SB2 and SB3. This evidence, combined with previous structural studies of the ILR1-reactive molecule, indicates that the SB gene product has the structure of an Ia-like molecule. Furthermore, the serologic analysis of HLA mutant cell lines with this monoclonal antibody supports the inference made from previous studies using cellular typing (24, 27, 28) that there are two HLA regions centromeric to HLA-B that control expression of Ia-like molecules: a region toward HLA-B that controls expression of HLA-DR, and a region toward GLO that controls expression of SB. These data provide an initial correlation between a triad of findings: the SB antigens function as immunogens in human cellular immune responses, they are encoded by a locus in the HLA region that is distinct from *HLA-DR*, and they are present on an Ia-like molecule. Thus, SB, like HLA-DR, appears to be a human equivalent of the murine Ia molecules, and may therefore be important in regulation of human immune responses.

Materials and Methods

Monoclonal Antibodies. Murine monoclonal antibodies I2, ILR1 (18, 26), and L227 (29) were grown in vitro or as ascites as previously described. The reactivity of cells with monoclonal antibodies was analyzed by indirect immunofluorescence measured by flow microfluorometry (FMF) using a fluorescence-activated cell sorter (FACS II, B-D FACS Systems, Becton, Dickinson & Co., Sunnyvale, CA) as described (30, 31). To optimize comparison of data collected at different linear electronic amplifications, fluorescence units (adjusted to gain 16) were calculated according to the formula: FU = (median fluorescence channel − 10) × 16/gain. The measurement of fluorescence units from cells incubated in the presence of the monoclonal antibody followed by fluorescent reagent [goat $F(ab')_2$ anti-mouse IgG(H+L), N. L. Cappel Laboratories, Cochraneville, PA] was corrected by subtraction of fluorescence units measured on the same cell population incubated with the fluorescent reagent alone. All reagents were pretested and subsequently used at saturating concentrations. Cells were scored as ILR1-negative if they had <40 fluorescent units of binding; all ILR1-positive donors had >150 fluorescent units of binding.

Cellular Studies. Primed lymphocyte typing for SB antigens was performed as previously described on peripheral blood mononuclear cells (23) and lymphoblastoid B cell lines (LCL) (25); serotyping of cells for DR antigens was kindly performed by Dr. R. Duquesnoy (Blood Center of Southeastern Wisconsin, Milwaukee, WI) using the standard National Institutes of Health (NIH) lymphocytotoxicity technique on peripheral blood B cells. LCL for the panel studies were established from peripheral blood lymphocytes using Epstein-Barr virus transformation as previously described (32). For antibody inhibition of proliferation, the routine 3-d primed lymphocyte-typing assay (23) was modified as follows. Ascites was diluted 1:25 into medium (RPMI 1640 with glutamine [Grand Island Biological Co., Grand Island, NY] with added penicillin [10 U/ml], streptomycin [10 μg/ml], and 15% heat-inactivated normal pooled human heparinized plasma). Diluted ascites or undiluted culture supernatant (25 μl) was added to flat-bottomed microtiter plates. Responder cells (2×10^4) and irradiated peripheral blood mononuclear stimulator cells (1×10^5) were each added in 25 μl of media. Cultures were established in triplicate and results are expressed as the geometric mean cpm of [^{3}H]thymidine incorporation.

Mutant Cell Lines. Cloned mutant cell lines were established using gamma irradiation and serologic negative selection according to the general procedures previously described (33).[2] The "DR-null" mutants were derived by negative selection from a mutant line (721.45.1) from

[2] R. DeMars, C. Chang, R. Rudersdorf, and H. T. Orr. Dissection of the human major histocompatibility complex with gamma ray-induced mutations in lymphoblastoid cells. Manuscript submitted for publication.

which an entire HLA haplotype had been physically deleted. A DR-specific monoclonal antibody L243 (29) was used to isolate DR-null mutants after gamma irradiation of line 721.45.1. The resulting mutants had lost expression of cell surface molecules detectable by antibody L243.

Statistics and Nomenclature. Overall statistical correlations of DR or SB and ILR1 reactivity were made using chi-square analysis. Comparisons of individual DR or SB alleles and ILR1 reactivity were made using the Fisher's exact test. Standard conventions have been used for genetic nomenclature (34).

Results

Population Studies of the Association between ILR1 Reactivity and HLA-DR and SB. Original characterization of the ILR1 antibody indicated that it detected a polymorphism which did not correlate with HLA-DR in the Caucasian population (18, 26). FMF analysis of the binding of ILR1 to our panel of 47 LCL (Table I): (*a*) demonstrates that ILR1 reactivity was observed with 66% of our cells; (*b*) confirms the lack of overall association with HLA-DR phenotype ($P = 0.33$); (*c*) demonstrates a strong overall association between cells' reactivity with ILR1 and their SB phenotype ($P < 0.003$). These findings strongly suggest that ILR1 might recognize determinants related to SB, but also demonstrate that ILR1 reactivity is not limited to a single allele of SB.

Analysis of associations between ILR1 and individual alleles of DR and SB (Table I) indicates that ILR1 binds to all donors' cells that express SB2, SB3, and DR5. Although the data achieve statistical significance only for SB2, they are consistent

TABLE I

Population Study of the Correlation between HLA-DR and SB and Binding of Monoclonal Antibody ILR1

Antigen	ILR1 + donors ($n = 31$)*		ILR1 − donors ($n = 16$)*	
	Number with antigen	Percent with antigen	Number with antigen	Percent with antigen
SB2‡	12*	39	0	0
SB3	8	26	0	0
SB blank	6	19	1	6
SB1	6	19	6	38
SB4	12	39	11	69
SB5	1	3	4	25
DR1‡	5	16	2	13
DR2	11	35	6	38
DR3	14	45	7	44
DR4	7	23	2	13
DR5	7	23	0	0
DR7	5	16	6	38
DR blank	1	3	0	0

* Binding of ILR1 to LCL was ascertained by FMF.

‡ The statistical significance (by chi square analysis) of the overall correlation between ILR1 and SB is highly significant ($P < 0.003$) but of the overall correlation between ILR1 and the six well-defined DR alleles is not significant ($P = 0.33$).

with the interpretation that ILR1 binds to all DR5, SB2, and SB3 molecules. The implication that ILR1 may bind to DR5 molecules is an interesting one, supported by other data (C. Hurley and S. Shaw, manuscript in preparation), but will not be documented further in this study as it is tangential to the primary focus, the SB antigens. More detailed information on the association between SB phenotype and ILR1-reactivity is represented in Fig. 1. For ease of visualization, donors are represented on either side of the figure's axis of symmetry. Because the DR5 molecule appears to bind ILR1, donors expressing DR5 are distinguished in the figure; however, none of the conclusions relating to SB are changed by inclusion or exclusion of those donors. All donors' cells expressing SB2 or SB3 also bind ILR1, suggesting that the SB2 and SB3 molecules bind ILR1. The remaining 25 donors' cells (which are negative for SB2 and SB3) provide information on ILR1 binding to other alleles. Of these, the seven that express two defined SB alleles (SB1,4; SB1,5; and SB4,5) are the most informative; all of them fail to bind ILR1, suggesting that SB1, SB4, and SB5 molecules lack the ILR1-reactive epitope. This interpretation is also supported by the observation that most of the donors (8/12) that express SB1 only (SB1,1 or SB1,blank) or SB4 only (SB4,4 or SB4,blank) fail to bind ILR1. Thus all the data are consistent with the hypothesis that ILR1 binds to SB2 and SB3 molecules but not to SB1, SB4, or SB5 molecules. However, ILR1 also binds to six donors cells that lack SB2, SB3, and DR5; although not inconsistent with the foregoing hypothesis, ILR1 binding to those six donors' cells is not explained by it and requires an additional hypothesis. Either the ILR1-reactive epitope is also present on some SB blank molecules, or there are other polymorphic molecules distinct from SB and DR that are also able to bind ILR1 (see Discussion).

ILR1 Inhibits Proliferation of SB-specific but not DR-specific Primed T Cells. To test more

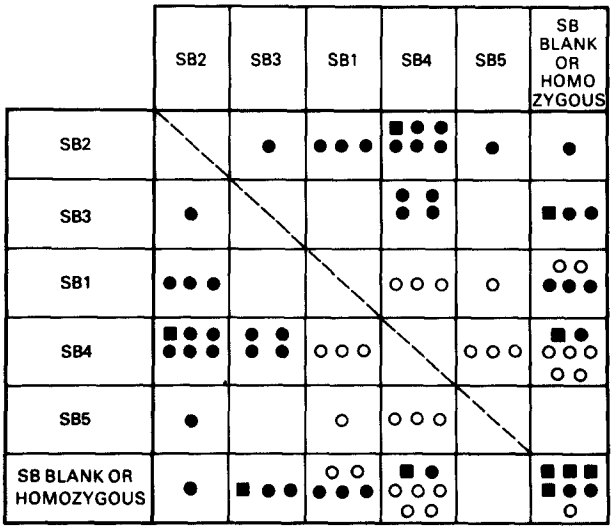

FIG. 1. Association between ILR1-reactivity and the SB phenotype of 47 donors' cells. Binding of ILR1 to LCL was ascertained by FMF. Cells that bind ILR1 (●), cells that express DR5 (all of which bind ILR1) (■) and those that do not bind ILR1 (○) are plotted according to their SB antigen phenotype. Note that there is an axis of symmetry; for ease of visualization all donors are represented by a symbol on either side of that axis, except the donors typing for SB blank only, whose cells are on that axis.

directly the relevance of ILR1-reactive molecules to the antigenic moieties defined by cellular typing, we tested the ability of ILR1 to block SB-specific T cell recognition. ILR1 was added during secondary proliferative responses of primed T cells responding to each of the five known SB antigens; responses to each of two DR antigens were included as controls (Table II). Addition of ILR1 reduced the proliferative responses to two of the five SB antigens (SB2 and SB3) by at least 65%, but did not inhibit the proliferative responses to the other three SB antigens or to the two DR antigens tested. For both of the inhibited responses (SB2 and SB3) ILR1 binds only to the stimulator cells, not to the responding cells; thus, inhibition occurs by a mechanism related to binding to the stimulator, not the responder cells. Furthermore, this inhibition is antigen specific; although ILR1 binds to the stimulator cells (from donor B17) that were used to stimulate both the DR2- and SB2-specific responses, it only inhibited the response to the SB2 antigen, not the DR2 antigen.

The results of another representative inhibition experiment (Table III) strengthen the evidence that ILR1 causes antigen-specific inhibition of T cell proliferation by binding to the stimulator cell. As in the previous experiment, the conclusions are that: (*a*) SB2- and SB3-specific responses are inhibited (by >70%), but SB1-, SB4-, SB5-, DR1-, and DR2-specific responses are not; (*b*) the inhibition operates at the level of the stimulator cell, as it occurs in combinations where stimulator but not responder cells bind ILR1; and (*c*) this inhibition is antigen specific, as inhibition of the SB2-specific response to cell Y2 is not accompanied by inhibition of the SB1-specific response, for which Y2 was also used as a stimulator cell. This experiment differs from

TABLE II

Selective Inhibition of Secondary Lymphocyte Proliferation by the Monoclonal Antibody ILR1

Stimulating antigen	Percent inhibition	ILR1 reactivity of cells from:		Proliferation in the presence of antibody*	
		Responder	Stimulator	Control‡	ILR1
				cpm	
SB2	78%	−	+	103.6	22.6
SB3	65%	−	+	46.3	16.1
SB1	5%	+	+	145.6	138.5
SB4	10%	+	−	120.8	108.1
SB5	8%	+	−	45.9	42.0
DR1	13%	+	+	37.0	32.3
DR2	0%	+	+	52.4	52.3

The effect of addition of ILR1 antibody on proliferative responses to SB and DR antigens. The combinations of donors used to generate the SB- and DR-specific primed cells are identical to those described previously (refer to refs. 22, 23 in which the following donors and priming combinations are described; the donor combination used to generate the SB1-primed cells used in this experiment was S11/H9, for SB2, F2/B17, for SB3, W7/M14, for SB4, S11/PM1, for SB5, B17/F2, for DR1, H9/K4, and for DR2, F1/B17); in each combination, the donors are identical for all HLA antigens (HLA-A, -B, [-C], -D, -DR, MB, MT, SB), except the one desired for priming.

* cpm expressed as cpm × 10^{-3}.

‡ The control antibody was I2, a DR-specific anti-Ia monoclonal antibody (18).

TABLE III

Selective Inhibition of Secondary Lymphocyte Proliferation by the Monoclonal Antibody ILR1

Stimulating antigen	Percent inhibition with:		ILR1 reactivity of cells from:		Proliferation in presence of antibody:*		
	ILR1	L227	Responder	Stimulator	Control‡	ILR1	L227
SB2	97	67	−	+	22.8	0.6	7.4
SB3	71	45	+	+	33.7	9.8	18.5
SB1	−13	89	+	+	35.2	39.9	3.9
SB4	−10	ND	+	−	71.2	78.6	ND
SB5	−37	56	+	−	56.8	77.8	24.7
DR1	8	9	+	+	22.5	20.8	21.4
DR2	−10	36	+	+	11.4	12.6	9.2

The effect of addition of ILR1 antibody on proliferative responses to SB and DR antigens. As in Table II, the combinations of donors used to generate the SB- and DR-specific primed cells are identical to those described previously (22, 23; for reference to those citations the donor combination used to generate the SB1-primed cells was FB6/FB11, for SB2, W7/H9, for SB3, PM1/M14, for SB4, H9/M14, for SB5, H9/S11, for DR1, H9/K4, and for DR2, F1/B17); in each combination, the donors are identical for all HLA antigens (HLA-A, -B, [-C], -D, -DR, MB, MT, SB), except the one desired for priming. The restimulating cells used in this experiment were Y2 (A26,32,B41,52,DR5,7,SB1,2) for SB1 and SB2, E2 (A3,B7,DR1,4,SB3) for SB3, FC4 (A2,B7,DR2,SB4,5) for SB4 and SB5, L2 (A26,31,B14,38,DR1,4,SB2,4) for DR1, and M8 (A2,B8,DR2,3,SB3) for DR2.

* cpm expressed as cpm × 10^{-3}

‡ The control antibody was I2.

the previous one in three features of its design. First, the primed cells responding to each of the SB antigens were derived from a different combination of responder and stimulator cells (the second of the two original donor combinations used to define these antigens; see 22, 23); therefore, the inhibition seen for SB2- and SB3-specific primed cells can be attributed to their antigen specificity and not to other attributes of the donor combinations. Second, although the primed cells used in these experiments were generated in carefully HLA-matched donor combinations and have proven to be functionally monospecific in extensive proliferation studies, a further precaution was taken to restrict the antigens recognized: the restimulating cells were chosen to be HLA-mismatched with the primary stimulating cell for all but the relevant HLA antigen (e.g., for SB2, cells from donor W7 [A1,2, B7,8, DR2,3, SB1,4], which had been primed against cells from donor H9 [A1,2, B7,8, DR2,3, SB1,2] and were restimulated during this assay with cells from donor Y2 [A26,32, B41,52, DR5,7, SB1,2]). This further refinement of specificity of the responding T cells did not alter the inhibition observed. Third, the possibility was considered that SB2- and SB3-specific responses might be nonspecifically more susceptible to all inhibitory effects of monoclonal antibodies. As a control for this possibility, an anti-Ia monoclonal antibody (L227) was included that had been seen previously to partially inhibit T cell proliferative responses to many specificities. The SB2- and SB3-specific proliferative responses were inhibited no more (and perhaps less) by monoclonal antibody L227 than were the proliferative responses specific for the other SB antigens.

Genetic Mapping of ILR1 Reactivity in HLA-mutant Cell Lines. Previous studies both of recombinant families (24) and of mutant cell lines (25) indicated that HLA-DR and SB were encoded by separate loci in the human MHC. If ILR1 recognizes an epitope on the same polypeptide as that detected by typing with cellular reagents, then the

genetics of ILR1 reactivity should parallel those of the SB antigens defined by cellular typing. This prediction was explored using panels of mutant cell lines that had been established from a parent LCL expressing the HLA haplotypes (*A*1*, *B*8*, *DR*3*, *SB*4*, *GLO*2*), and (*A*2*, *B*5*, *DR*1*, *SB*2*, and *GLO*1*). Pilot studies indicate that an ILR1-reactive molecule was encoded by a locus of the *SB*2* haplotype, as would be predicted from the studies described above. Of the mutant lines available, 13 were chosen that had lost *DR*1* from one HLA haplotype and an unknown amount of genetic material centromeric to *DR*1*; these lines were assayed for expression of the SB2 antigen (as detected by cellular typing) and expression of the ILR1-reactive molecule (Table IV, Fig. 2). All seven independent lines that retained the SB2 antigen also retained their ILR1-reactivity (type 1 and type 3); none that lost SB2 (type 2) retained ILR1 reactivity ($P < 0.002$, two-tailed Fisher's exact test). The reactivity of ILR1 with mutant cell lines that have lost expression of their DR1 molecule encoded

TABLE IV

Correlation between ILR1 Reactivity and SB2 Expression on Mutant Cell Lines that Have Lost DR1

Mutant line number	Primed cells used to identify SB2*		Monoclonal Ab reactivity	
	SB2A	SB2B	ILR1	I2 (DR)
Parent	100*	100*	100‡	100‡
Type 1§				
.51	52	52	150	109
.52	39	58	155	599
Type 2				
.77	7	6	1	82
.78	8	6	1	131
.113	9	7	1	128
.116	3	4	1	192
.120	6	4	0	187
.127	19	7	1	229
Type 3				
.81.3	125	85	184	0
.137.1	143	117	273	0
.82.4	62	83	189	0
.84.5	98	98	200	0
.101.1	101	83	167	0

* The primed cell reagents used to detect the SB2 antigens are identical to those previously described (22, 23); SB2A is generated by donor W7 anti-H9 and SB2B by donor F2 anti-B17. The proliferative data are expressed in terms of relative response, i.e., responses induced by the mutant stimulator cell compared with that induced by the parent line. Absolute cpm for the responses was 29,750 for the SB2A-primed cells and 76,880 for the SB2B-primed cells.

‡ Antibody-binding data are expressed as percentage of the expression on the parent line, as measured by FMF. The level of expression on the parent line was 5684 fluorescence units for I2 and 3964 fluorescence units for ILR1.

§ See Fig. 2 for schematic representation showing different mutant types. All type 1 and type 2 mutants had lost expression of the A2 and B5 markers on that haplotype. Two of the type 2 mutants had lost GLO*1 marker on that haplotype.

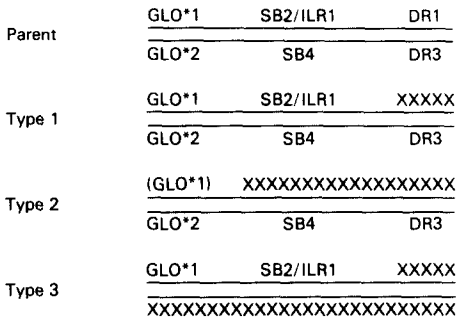

FIG. 2. Schematic of the *HLA-DR/GLO* segment of the HLA haplotypes of the HLA-deletion mutants analyzed. For data, see Table IV. Of mutants that lost only part of one HLA haplotype, some had lost DR1 but not SB2 or ILR1 (type 1), whereas others had lost DR1 as well as SB2 and ILR1 (and two had lost expression of GLO*1) (type 2). Of mutants that lost the entire DR3 haplotype and DR1, none had lost SB2 or ILR1 (type 3). No mutants were identified which had lost SB2 but not ILR1-reactivity, or vice versa.

on the *SB*2*-containing haplotype demonstrates that there are at least two loci controlling expression of products that bind anti-Ia monoclonal antibodies. Because the type 1 mutants had lost expression of genes in the segment *HLA-A*2/HLA-B*5/DR*1*, the retention of ILR1-reactivity in these mutants indicates that the gene that controls ILR1-detected polymorphism does not map between *HLA-A* and *DR*.

These data are most dramatic for the DR-null mutant lines (Fig. 2, type 3), which have lost the entire opposite HLA haplotype. All five of these lines have lost reactivity with a monoclonal antibody detecting one class of Ia molecules (including HLA-DR) but retain reactivity with ILR1. Thus, the ILR1 reactive molecule that was previously shown to be distinct from DR immunochemically (18, 26), is shown by these studies to be controlled by a locus distinct from those controlling HLA-DR.

Discussion

Three lines of evidence presented in this report indicate that the ILR1 antibody binds to some alleles of the SB molecule: (*a*) the polymorphism of ILR1-reactivity correlates with SB2 and SB3 antigens in the population; (*b*) T cell proliferative response to SB2 and SB3 are specifically inhibited by ILR1; and (*c*) the expression of ILR1 is exactly concordant with the expression of SB2 in a panel of HLA deletion mutant LCL.

The studies of antibody inhibition are taken to indicate that ILR1-reactive molecules per se are responsible for inducing SB2- and SB3-specific proliferation. The simplest mechanism for explaining the observed inhibition is antibody-mediated steric hindrance of interaction between the T cells and the SB molecule. This seems more plausible than other possible mechanisms, such as one by which ILR1 could induce suppression. This is conceivable, but no experimental evidence has been found for suppression in this system (S. Shaw, unpublished observations), and this inhibition differs in two important ways from the suppression-induction previously reported (35) for anti-Ia antibodies: it is antigen-specific, and the inhibition is observed in a secondary rather than a primary response. It is also possible that the ILR1-reactive molecule is distinct from SB but has a unique steric relationship to SB which accounts for the inhibition. This is an unattractive hypothesis as this postulated molecule

would also have to (*a*) have a polymorphism in the population indistinguishable from that of SB (to explain the population association); (*b*) be controlled by a locus located close to SB (to explain the mutant data); and (*c*) have a unique relationship on the cell surface with SB2 and SB3 but not, for example, with SB1 or DR2 (because concurrent responses to those antigens on the same cell are not inhibited).

Thus, several lines of evidence indicate that ILR1 binds to an epitope on the SB2 and the SB3 molecules. In addition, the results of population studies in this report, when combined with those previously published (18), demonstrate a correlation between DR5 and ILR1 reactivity ($P < 0.004$, Fisher's exact test) that retains statistical significance ($P < 0.05$) even after correction for the number of antigens analyzed. Several other lines of evidence support the conclusion that ILR1 binds to DR5 but to no other allotypic forms of HLA-DR (C. Hurley and S. Shaw, manuscript in preparation). Thus, ILR1 appears to bind SB2, SB3, and DR5. There are six donors, however (Fig. 1), whose cells bind ILR1 despite the fact that they do not express SB2, SB3, and DR5. This could indicate that ILR1 binds not only to some DR molecules and some SB molecules, but also to some products of another gene. There is no need to postulate involvement of a third gene product, though; because all six donors with unexplained ILR1 reactivity have at least one undefined SB allele, they may express another allotypic form of the SB molecule (e.g., a future SB6 or SB7) that also binds ILR1. This hypothesis is consistent with emerging evidence for a new SB allele that is crossreactive with SB4 but that, unlike SB4, binds ILR1 (S. Shaw, unpublished observations).

Thus, the ILR1-binding epitope appears to be shared by some SB and DR molecules. Monoclonal antibodies have identified polymorphic epitopes shared by distinct but homologous murine Ia molecules: IA and IE (36). The sharing of such an epitope between DR and SB would appear to reflect extensive homology between these two distinct gene products. Another study of monoclonal antibody inhibition of proliferation also suggests that SB and DR share serologically defined determinants.[3] In that study, some monoclonal antibodies specific for monomorphic epitopes on human Ia molecules were able to inhibit proliferation of T cells specific for all HLA-DR and SB determinants tested. Data from these two studies are consistent with the hypothesis proposed previously (22) that *SB* and *DR* genes are derived from duplication of an ancestral gene(s).

Although ILR1 is not exclusively SB specific, data indicate that ILR1 can be used to identify SB molecules in informative donors; i.e., that are positive for SB2 or SB3 but negative for DR5. Such an approach is subject to the caveat that ILR1, like all other monoclonal antibodies, may also bind other unidentified gene products. Previous immunochemical studies (18, 26) on cells now known to be informative for ILR1 binding to SB indicate that ILR1 precipitates a glycoprotein composed of two polypeptide chains with molecular weights of 29,000 and 34,000. Preliminary results of further structural studies of the ILR1-reactive molecule indicate that it has an alpha chain amino acid sequence that is similar or identical to the HLA-DR alpha chain, but has a novel beta chain (C. Hurley and J. D. Capra, personal communication). These results support the hypothesis that SB molecules are typical Ia-like

[3] G. Pawalec, S. Shaw, A. Ziegler, C. Muller, and P. Wernet. Differential inhibition of HLA-DR- or SB-directed secondary lymphoproliferative responses with monoclonal antibodies detecting human Ia-like determinants. *J. Immunol.* In press.

molecules, as proposed previously (22) on the basis of the function of SB determinants in allogeneic T cell responses and their tissue distribution. The only approach that can demonstrate with certainty that the functions of the SB antigens are served by epitopes on Ia-like molecules is the direct demonstration of SB-specific responses to those molecules after their isolation and purification.

The availability of a serological reagent to define SB2 and SB3 antigens in informative donors allows refinement of the previous conclusions on the tissue distribution of the SB determinants implied in studies of target cell susceptibility to cell-mediated lysis (22). In such donors, the ILR1-reactive molecule was serologically identifiable on peripheral B cells, macrophages, and particularly well on B cell LCL, but not on resting T cells (26). ILR1 studies resolve an uncertainty of cellular studies by demonstrating that activated T cells express SB antigen, as they do DR antigens (37, 38).

Studies on the mutant cell lines corroborate the close genetic relationship between ILR1 reactivity and SB expression—i.e., that, so far, they are indistinguishable. Those studies also provide direct serologic confirmation that there are two HLA regions centromeric to HLA-B that control expression of Ia-like molecules. This had been suggested by family studies of alloantisera (7, 21) and can be inferred from family studies using cellular typing techniques (24, 27, 28). Mapping of the loci controlling expression of these Ia-like antigens is facilitated by the mutants. Because ILR1 reactivity is preserved in mutants that have lost expression of genes in the *HLA-A/DR* segment (the type 1 mutants), the gene controlling its polymorphism does not map to that region. Because polymorphism of ILR1 has been previously mapped to the *HLA-DR* side of a *HLA-B/DR* recombination (18), ILR1 reactivity appears to be controlled by a least one gene that maps centromeric to *HLA-DR*. Thus, in the region bounded by *HLA-B* and *GLO* (often referred to by the vague term D region), there is a subregion toward HLA-B including a locus that controls expression of HLA-DR (defined serologically by alloantisera and monoclonal antibodies L243 and I2), and a subregion toward GLO that includes a locus that controls expression of SB (defined serologically by monoclonal antibody ILR1). Mutagenesis of LCL promises to continue as a powerful tool in dissecting out the genetics of HLA, as there is already preliminary evidence for serological and cellular definition of products of genes mapping to a third subregion of this genetic segment (*HLA-B/GLO*), which are expressed on some but not other mutants (R. Duquesnoy, F. H. Bach, and R. DeMars, unpublished observations).

The existence of this antibody, which detects the SB molecule, resolves uncertainty as to whether the SB antigens are readily identifiable serologically. Concurrent with these studies, evidence is emerging that alloantisera can be identified that bind to HLA determinants highly associated in the population with SB polymorphism.[4] Development of serological tools that identify the SB gene product will facilitate further studies of the biological relevance of this recently discovered human Ia-like gene product.

Summary

The secondary B cell (SB) antigens are polymorphic HLA-linked antigens on human B cells and macrophages that are identified by primed T cell responses but

[4] A. Van Leeuwen, A. Termijtelen, S. Shaw, and J. J. Van Rood. The recognition of a polymorphic monocyte antigen in HLA. Manuscript submitted for publication.

are genetically distinct from the HLA-DR, MB, and MT antigens. Serologic identification of the SB molecule, using the monoclonal antibody ILR1, now makes it possible to correlate the function of these determinants in human T cell recognition with an Ia-like molecular structure and a genetic locus that marks a new HLA subregion. Three lines of evidence indicate that the ILR1 molecule identifies an epitope on some alleles of the SB gene: (*a*) the polymorphism of ILR1-reactivity in the population correlates with SB2, SB3; (*b*) T cell proliferative response to SB2 and SB3 are specifically inhibited by ILR1; and (*c*) ILR1 reactivity is exactly concordant with the expression of SB2 in a panel of HLA-deletion mutant lymphoblastoid cell lines. Together with previous studies, these results indicate that the SB antigens are on Ia-like molecules. Furthermore, the serologic studies of HLA-deletion mutant cell lines demonstrate that there are two HLA regions centromeric to HLA-B controlling expression of Ia-like molecules: a region toward HLA-B that controls expression of HLA-DR, and a region toward GLO that controls expression of SB.

We thank the staff of the NIH blood bank for leukapheresing donors, S. Sharrow and D. Stephany for performing the FMF analysis, C. Chang and R. Rudersdorf for production of the mutant LCL, the staff of the Blood Center of Southeastern Wisconsin for serotyping donors' cells, and Dr. W. Terry, Dr. G. Shearer, Dr. B. Biddison, Dr. D. Sachs, and Dr. D. Singer for valuable discussion.

Received for publication 27 April 1982.

References

1. Uhr, J. W., J. D. Capra, E. S. Vitetta, and R. G. Cook. 1979. Organization of the immune response genes. Both subunits of murine I-A and I-E/C molecules are encoded within the I region. *Science (Wash. D. C.).* **206:**292.
2. Benacerraf, B. 1981. Role of MHC gene products in immune regulation. *Science (Wash. D. C.).* **212:**1229.
3. Barnstable, L. J., and E. A. Jones. 1978. Isolation, structure and genetics of the HLA-A, -B, -D, and -DRw (Ia) antigens. *Br. Med. J.* **32:**241.
4. Amos, D. B., and D. D. Kostyu. 1980. HLA—a central immunological agency in man. *Adv. Hum. Genet.* **10:**137.
5. Van Rood, J. J., R. R. P. De Vries, and B. A. Bradley. 1981. Genetics and biology of the HLA system. *In* The Role of the MHC in Immunology. M. E. Dorf, editor. Garland Publishing, Inc., New York. 59–113.
6. Duquesnoy, R. J., and M. Marrari. 1980. MB system. *In* Histocompatibility Testing 1980. P. I. Terasaki, editor. UCLA Tissue Typing Laboratory, Los Angeles. 552–555.
7. Park, M. S., and P. I. Terasaki. 1980. Second DR locus. *In* Histocompatibility Testing 1980. P. I. Terasaki, editor. UCLA Tissue Typing Laboratory, Los Angeles. 578–582.
8. Park, M. S., P. I. Terasaki, S. Nakata, and D. Aoki. 1980. Supertypic groups: DR, MT1, MT2, and MT3. *In* Histocompatibility Testing 1980. P. I. Terasaki, editor. UCLA Tissue Typing Laboratory, Los Angeles. 854–857.
9. Katagira, M., H. Ikeda, N. Maruyama, J. Moriuchi, A. Wakisaka, S. Kimura, M. Aizawa, and K. Itakura. 1979. Evidence for two B cell alloantigen loci in the HLA-D region. *Immunogenetics.* **9:**335.
10. Tosi, R., N. Tanigaki, D. Centis, G. B. Ferrara, and D. Pressman. 1978. Immunological dissection of human Ia molecules. *J. Exp. Med.* **148:**1592.
11. Corte, G., F. Calabi, G. Domiani, A. Bargellesi, R. Tosi, and R. Sorrentino. 1981. Human Ia molecules carrying DC1 determinants differ in both alpha and beta subunits from Ia

molecules carrying DR determinants. *Nature* (*Lond.*). **292:**537.

12. Shackelford, D. A., D. L. Mann, J. J. Van Rood, G. B. Ferrara, and J. L. Strominger. 1981. Human B-cell alloantigens DC1, MT1, and LB12 are identical to each other but distinct from the HLA-DR antigen. *Proc. Natl. Acad. Sci. U. S. A.* **78:**4566.
13. Shackelford, D. A., and J. L. Strominger. 1980. Demonstration of structural polymorphism among HLA-DR light chains by two-dimensional electrophoresis. *J. Exp. Med.* **151:**144.
14. Charron, D. J., and H. O. McDevitt. 1979. Analysis of the HLA-D region-associated molecules with monoclonal antibody. *Proc. Natl. Acad. Sci. U. S. A.* **76:**6567.
15. Markert, M. L., and P. Cresswell. 1980. Polymorphism of human B-cell alloantigens: evidence for three loci within the HLA system. *Proc. Natl. Acad. Sci. U. S. A.* **77:**6106.
16. Quaranta, V., M. A. Pellegrino, and S. Ferrone. 1981. Serologic and immunochemical characterization of the specificity of four monoclonal antibodies to distinct antigenic determinants expressed on subpopulations of human Ia-like antigens. *J. Immunol.* **126:**548.
17. Goyert, S. M., and J. Silver. 1981. Isolation of I-A subregion like molecules from subhuman primates and man. *Nature* (*Lond.*). **294:**266.
18. Nadler, L. M., P. Stashenko, R. Hardy, K. J. Tomaselli, E. J. Yunis, S. F. Schlossman, and J. M. Pesando. 1981. Monoclonal antibody identifies a new Ia-like (p29,34) polymorphic system linked to the HLA-D/DR region. *Nature* (*Lond.*). **290:**591.
19. Kratzin, H., C.-Y. Yang, H. Gotz, D. Pauly, S. Kolbel, G. Egert, F. P. Thines, P. Wernet, P. Altevogt, and N. Hilschman. 1981. Primarstruktur menschlicher Histokompatibilitaetsantigen der klasse II. I. Mitteilung: aminosaeuresequenz der N-terminalen 198 reste der beta-kette des HLA-Dw2,2; DR2,2-alloantigens. *Hoppe-Seyler's Z. Physiol. Chem.* **362:**1665.
20. Duquesnoy, R. J., A. Zeevi, M. Marrari, and K. Halim. 1982. Immunogenetic analysis of the HLA-D region: serological and cellular detection of the MB system. *Clin. Immunol. Immunopathol.* In press.
21. Van Leeuwen, A., H. Festenstein, J. D'Amaro, C. Naverette, J. Awad, and J. J. Van Rood. 1980. A new B cell locus defined by a recombinant family. *In* Histocompatibility Testing 1980. P. I. Terasaki, editor. UCLA Tissue Typing Laboratory, Los Angeles. 853.
22. Shaw, S., A. H. Johnson, and G. M. Shearer. 1980. Evidence for a new segregant series of B cell antigens that are encoded in the HLA-D region and that stimulate secondary allogeneic proliferative and cytotoxic responses. *J. Exp. Med.* **152:**565.
23. Shaw, S., R. J. Duquesnoy, and P. L. Smith. 1981. Population studies of the HLA-linked SB antigens. *Immunogenetics.* **14:**153.
24. Shaw, S., P. Kavathas, M. S. Pollack, D. Charmot, and C. Mawas. 1981. Family studies define a new histocompatibility locus, SB, between HLA-DR and GLO. *Nature* (*Lond.*). **293:**745.
25. Kavathas, P., R. DeMars, F. H. Bach, and S. Shaw. 1981. SB: a new HLA-linked human histocompatibility gene defined using HLA-mutant cell lines. *Nature* (*Lond.*). **293:**747.
26. Nadler, L. M., P. Stashenko, R. Hardy, J. M. Pesando, E. J. Yunis, and S. F. Schlossman. 1981. Monoclonal antibodies defining serologically distinct HLA-D/DR related Ia-like antigens in man. *Hum. Immunol.* **1:**77.
27. Mawas, C., D. Charmot, M. Sivy, P. Mercier, M. L. North, and G. Hauptmann. 1978. A weak human MLR locus mapping at the right of a crossing over between HLA-D,Bf and GLO. *J. Immunogenet.* **5:**583.
28. Mawas, C., D. Charmot, and P. Mercier. 1980. Split of HLA-D into two regions alpha and beta by a recombination between HLA-D and GLO. I. Study in a family and primed lymphocyte typing for determinants coded by the beta region. *Tissue Antigens.* **15:**458.
29. Lampson, L. A., and R. Levy. 1980. Two populations of Ia-like molecules on a human B cell line. *J. Immunol.* **125:**293.
30. Sharrow, S. O., K. Ozato, and D. H. Sachs. 1980. Phenotypic expression of I-A and I-E/C subregion determinants on murine thymocytes. *J. Immunol.* **125:**2263.

31. Mathieson, B. J., S. O. Sharrow, P. S. Campbell, and R. Asofsky. 1979. An Lyt differentiated subpopulation of thymocytes detected by flow microfluorometry. *Nature (Lond.)*. **277:**478.
32. Bird, A. G., S. M. McLachlan, and S. Britton. 1981. Cyclosporin A promotes spontaneous outgrowth in vitro of Epstein-Barr virus induced B-cell lines. *Nature (Lond.)*. **289:**300.
33. Kavathas, P., F. H. Bach, and R. DeMars. 1980. Gamma ray-induced loss of expression of HLA and glyoxylase I alleles in lymphoblastoid cells. *Proc. Natl. Acad. Sci. U. S. A.* **77:**4251.
34. Shows, T. B. 1979. Conventions on genetic nomenclature. *Cytogenet. Cell Genet.* **25:**96.
35. Broder, S., D. L. Mann, and T. A. Waldmann. 1980. Participation of suppressor T cells in the immunosuppressive activity of a heteroantiserum to human Ia-like antigens (p23,30). *J. Exp. Med.* **151:**257.
36. Pierres, M., C. Devaux, M. Dosseto, and S. Marchetto. 1981. Clonal analysis of B- and T-cell responses to Ia antigens. I. Topology of epitope regions on I-A^k and I-E^k molecules analyzed with 35 monoclonal antibodies. *Immunogenetics.* **14:**481.
37. Ko, H.-S., S. M. Fu, R. J. Winchester, D. T. Y. Yu, and H. G. Kunkel. 1979. Ia determinant on stimulated human lymphocytes. Occurence on mitogen- and antigen-activated T cells. *J. Exp. Med.* **150:**246.
38. Reinherz, E. L., P. C. Kung, J. M. Pesando, J. Ritz, G. Goldstein, and S. F. Schlossman. 1979. Ia determinants on human T cell subsets defined by monoclonal antibody. Activation stimuli required for expression. *J. Exp. Med.* **150:**1472.