

ONTOGENY OF B LYMPHOCYTES

III. *H-2* LINKAGE OF A GENE CONTROLLING THE RATE OF APPEARANCE OF COMPLEMENT RECEPTOR LYMPHOCYTES

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In the previous papers of this series, we have reported that substantial numbers of lymphocytes bearing surface immunoglobulin (Ig) are found in the spleens of mice by the 1st day of life,¹ whereas lymphocytes bearing complement (C) receptors cannot be detected until later (1). Since surface Ig and the C receptor are both found on lymphocytes of the bursal-equivalent (B)² line (2, 3), this sequential appearance of surface Ig and C receptors represents a pattern of B-lymphocyte differentiation.

In AKR mice, the lag between the appearance of surface Ig and C receptors is relatively short (1) so that C receptor lymphocytes (CRL) can be detected by 1 wk of age and are a substantial fraction (27%) of the splenic lymphocyte population at 2 wk of age. In other strains, such as BALB/c, C57BL/6, and DBA/2, CRL occur in low frequency (<7%) at 2 wk of age, although adult animals of these strains have a normal frequency of CRL (25–30%) (1).

Analysis of CRL frequency at 2 wk of age in the progeny of (AKR × DBA/2)F₁ × DBA/2 backcrosses suggested that the rate of CRL appearance was under the control of two independent genes. Possession of the “high” allele of either gene appeared to lead to an intermediate or high CRL frequency at 2 wk of age (1). In this communication, we demonstrate that one of the genes controlling the rate of CRL appearance is linked to the mouse *H-2* complex. Mapping studies indicate that this gene is telomeric to the genes controlling serum substance (*Ss-Slp*) and centromeric to the *Tla* locus.

Materials and Methods

Mice.—AKR/J, DBA/2J, (AKR/J × DBA/2J)F₁ [AKD2], C57BL/6J, C57BL/10J, B10.A/SgSn, B10.D2/oSn, B10.D2/nSn, B10.BR/SgSn, C57BR/J, A/WySn, A.BY A.SW, and A.CA mice were obtained from Jackson Laboratories, Bar Harbor, Maine. B10.A(1R),

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²*Abbreviations used in this paper:* B, bursal equivalent line; CRL, C receptor lymphocytes; H, heavy; Ir, immune response; MHC, major histocompatibility complex; MLR, mixed lymphocyte responses.

B10.A(2R), B10.A(4R), B10.A(5R), (AKR \times C57BL/6) F_1 [AKB6], (AKB6 \times C57BL/6), and (AKD2 \times DBA/2) mice were bred at the National Institutes of Health, Bethesda, Md. A/JBoy [A/TL⁺], A-Tla^b [A/TL⁻], C57BL/6JBoy [B6/TL⁻], and C57BL/6-Tla^a [B6/TL⁺] mice were bred at the Sloan-Kettering Institute, New York.

Detection of CRL.—Mice were sacrificed at 13–17 days of age, spleens removed, and frequency of CRL measured as previously reported (1).

Histocompatibility Typing.—The *H-2* genotypes of backcross progeny were determined by cytotoxic testing on portions of the same spleen cell suspensions which were assessed for CRL. All antisera were produced by multiple weekly intraperitoneal injections of live lymphoid cells between appropriate strains of congenic mice. For progeny of (AKD2 \times DBA/2) matings, a B10.D2 anti-B10.BR antiserum was used to detect products of the *H-2^k* haplotype and a B10.BR anti-B10.D2 antiserum was used to detect products of the *H-2^d* haplotype. For progeny of (AKB6 \times B6) matings, a C57BL/10 anti-B10.A antiserum was used to detect products of the *H-2^k* haplotype (i.e., *K*-region *H-2^k* specificities) and a B10.A anti-C57BL/10 antiserum was used to detect products of the *H-2^b* haplotype. Cytotoxic assays were performed in microtiter “U” plates (Cooke Laboratory Products, Cooke Engineering Co., Alexandria, Va.) by the “2-stage” method and were read microscopically, all as previously described (4). The use of sera produced in congenic mice eliminates the possibility that serotyping might detect non-*H-2* specificities.

Determination of Ig Allotype.—Anti-G⁴H⁴ allotype antiserum was prepared in BALB/c mice immunized with washed *Bacillus proteus* immune agglutinates from AL/N mice (5). The G⁴H⁴ determinants are present on IgG (γ_{2a}) and IgH (γ_{2b}) myeloma proteins of the congenic C.AL9 mouse (6) and also in normal serum of strains of the a⁴ IgC_H linkage group which includes AKR but not DBA/2.

The anti-G⁴H⁴ antiserum was tested for precipitation in double gel diffusion tests with sera from progeny of (AKD2 \times DBA/2) matings as previously described (5). Detection of the presence of AKR allotype in the sera of the progeny was carried out at 6 wk of age on mice splenectomized at 2 wk of age for determination of splenic CRL frequency.

RESULTS

H-2 Linkage of a CRL Gene.—24 progeny of (AKD2 \times DBA/2) and (AKB6 \times C57BL/6) matings were splenectomized at 2 wk of age and CRL frequency and *H-2* type determined on cells from individual animals. In both crosses, F_1 mice from matings of a high CRL strain (AKR [*H-2^k*]) and a low CRL strain (DBA/2 [*H-2^d*] or C57BL/6 [*H-2^b*]) were mated with mice of that parental strain which has a low CRL frequency at 2 wk of age. In the cross of the AKD2 \times DBA/2, 3 of the 19 progeny from two litters were of low CRL type (<6.6% CRL), and all 3 of these mice were homozygous for *H-2^d*. An upper value of 6.6% for low CRL type was used in this analysis because this value is two standard deviations below the mean for CRL frequency in 2-wk old AKD2 mice and is higher than any observed value in DBA/2 mice (1). Similarly, in the cross of the AKB6 \times C57BL/6, one of the five progeny was of low CRL type and this mouse was an *H-2^b* homozygote (Table I). The probability that the results in the first cross (or summated results from both crosses) could have occurred by chance is <0.02 by chi-square testing. In view of our previous experiments suggesting that AKR and DBA/2 mice differ at two genetic loci governing rate of CRL appearance and of similar evidence that this is also true for AKR and C57BL/6 mice, the present result indicates

TABLE I
H-2 Linkage of CRL-1

	No.	<i>dd</i>	H-2 type	<i>dk</i>
1. AKD2 × DBA/2				
Low CRL mice	3	3		0
Intermediate and high CRL mice	16	2		14
	<i>P</i> < 0.02			
	No.	<i>bb</i>		<i>bk</i>
2. AKB6 × C57BL/6				
Low CRL mice	1	1		0
Intermediate and high CRL mice	4	2		2
	not significant			
3. Summated data				
	<i>P</i> < 0.02			

2-wk old progeny of F₁ × low CRL parent crosses were evaluated for CRL frequency. Data were analyzed by the Chi square test with Yates correction.

that one of these two genes is associated with the *H-2* complex. Possession of a single AKR *H-2^k* haplotype is sufficient to lead to a frequency of CRL at 2 wk of age which is greater than that observed in low CRL strains. We tentatively propose that this *H-2*-linked gene controlling CRL frequency in 2-wk old mice be termed *CRL-1*.

CRL Frequency in 2-wk Old Congenic Mice.—In order to explore more fully the role of genes associated with the *H-2* complex in the control of CRL appearance, we examined the frequency of CRL in 2-wk old mice in two congenic series. In the C57BL/10 series, the genetic background is derived from a “low CRL” strain, and the effect of the *H-2* complex derived from a group of other strains was evaluated. In the A/WySn series, the genetic background is derived from a strain with a CRL frequency intermediate between the low and high CRL strains, and the effect of *H-2* complexes from other strains was evaluated. In these experiments, we determined CRL frequency in the congenic mice, in the mice donating the “genetic background”, and, where possible, in the mice donating the *H-2* complex.

Table II shows that within the B10 congenic series, the C57BL/10 has a low CRL frequency (7%) at 2 wk of age, as do the B10.D2 (both old and new lines) and the B10.BR. On the other hand, the B10.A has an intermediate CRL level (14.5%). When CRL frequencies in mice donating the *H-2* complex are investigated, it is seen that the DBA/2 and the C57BR, which donated the *H-2^d* and the *H-2^k* chromosomes to the B10.D2 and B10.BR, respectively, have low CRL frequencies whereas the A/WySn, the donor of the *H-2^a* chromosome of the B10.A, has an intermediate CRL frequency. Thus, a congenic mouse (B10.A) deriving its *H-2* complex from an intermediate CRL

TABLE II
Frequency of CRL in 2-wk Old Congenic Mice

Strain	<i>H-2</i> type	CRL
		%
C57BL/10	<i>b</i>	7.6 ± 0.4
B10.A*	<i>a</i>	14.5 ± 0.5
A/WySn*	<i>a</i>	12.0 ± 0.5
B10.BR*	<i>k</i>	4.9 ± 0.5
C57BR*	<i>k</i>	3.8 ± 0.5
B10.D2*	<i>d</i>	6.0 ± 0.5
DBA/2*	<i>d</i>	4.9 ± 0.4
A/WySn	<i>a</i>	12.0 ± 0.5
A.BY	<i>b</i>	4.6 ± 0.5
A.SW	<i>s</i>	6.0 ± 0.9
A.CA	<i>f</i>	2.2 ± 0.6

* In the indicated pairs, the congenic strain received its *H-2* region from the other member of the pair.

strain (A/WySn) and the remainder of its genome from a low CRL strain (C57BL/10) has an intermediate CRL frequency at 2 wk of age. Similarly, mice which derive their *H-2* complexes from low CRL strains (DBA/2, C57BR, or C57BL/10 itself) and the remainder of their genome from a low CRL strain (C57BL/10) have low CRL frequencies at 2 wk of age. This provides additional evidence that a gene linked to the *H-2* complex is involved in the control of the rate of CRL appearance. It should be noted that the A/WySn and A/J are referred to as intermediate CRL strains because CRL frequency at 2 wk of age is 12.0 and 13.5%, respectively. This is clearly greater than the values for DBA/2, BALB/c, C57BL/6, C57BL/10, or C57BR, but lower than the value for AKR (~28%). In addition, it was noted that both C57BR and B10.BR strains, which are both *H-2^k*, were of low CRL type while the AKR, which is also *H-2^k*, is a high CRL strain. Since backcross evidence indicates that the *H-2^k* complex of the AKR is involved in control of CRL frequency, this would indicate that a high allele of *CRL-1* is linked to the *H-2^k* complex of the AKR strain while a low allele of *CRL-1* is linked to the *H-2^k* complex of the C57BR strain.

In the A/WySn series, the A.BY, A.CA, and A.SW mice are all low CRL strains, whereas the A/WySn is an "intermediate CRL" strain (Table II). Unfortunately, none of the strains donating the *H-2* complexes in this congenic series are available. Nonetheless, the demonstration that replacement of the *H-2^a* genotype in the A/WySn with either an *H-2^b*, *H-2^s*, or *H-2^f* geno-

type leads to a strain with a low 2-wk CRL frequency further substantiates the linkage of one of the CRL genes to the *H-2* complex.

CRL Frequency in 2-wk Old Mice with Recombinant H-2 Regions.—It is now recognized that the *H-2* complex comprises a relatively long segment of the genome, and several “marker” genes have been described and mapped within this complex (7, 8). The most centromeric (furthest to the left) region of the *H-2* complex is the *H-2K* region which controls the *K*-region serologic specificities. To the right of this are a series of immune response (*Ir*) genes including *Ir-1* and *Ir-IgA* which have not yet been aligned. Telomeric to this group of *Ir* genes is an additional *Ir* gene (*Ir-IgG*) which has been formally separated from the previously mentioned *Ir* genes. Further to the right are the genes controlling serum substance (*Ss*) and sex-limited protein (*Slp*). Most telomeric in the *H-2* complex itself is the *H-2D* region, which controls the *D*-region serologic specificities. To the right of the *H-2* complex, at a distance of about 1–2 map units is the *Tla* gene which controls the appearance of the TL antigen on thymocytes (9).

In order to more closely localized *CRL-1*, we studied the frequency of CRL in the spleens of 2-wk old congenic mice which possess *H-2* regions (or *H-2-T-la* regions) derived by recombination between low and intermediate CRL types. We initially utilized a series of three strains bearing *H-2* regions derived from recombination between *H-2^a* and *H-2^b* donors. These mice were the B10.A(2R), (4R), and (5R), respectively. The *H-2* complexes of these three recombinant strains are indicated in Table III. The B10.A(4R) mouse, which derived its *H-2K* and *Ir-1* genes from the *H-2^a* parent (an intermediate CRL type) and its *Ir-IgG*, *Ss-Slp*, and *H-2D* genes from the *H-2^b* parent (a low CRL strain) is a low CRL strain and thus resembles the donor of the “right” side of the *H-2* region. A similar linkage situation occurs in the B10.A(5R) mouse which derives its *H-2K*, *Ir-1*, and *Ir-IgG* genes from the low CRL (*H-2^b*) parent and its *Ss-Slp* and *H-2D* genes from the high CRL parent. The B10.A(5R) strain has a high frequency of CRL at 2 wk of age. This would indicate that *CRL-1* should be placed to the right of the site of the crossover events which gave rise to the *H-2^{hd}* and *H-2^{ic}* chromosomes. Finally, the B10.A(2R) mouse, which derived its *H-2K*, *Ir-1*, *Ir-IgG*, and *Ss-Slp* genes from the *H-2^a* parent and its *H-2D* gene from the *H-2^b* parent, has a low frequency of CRL at 2 wk of age like the donor of its *H-2D* region. This allows us to place *CRL-1* to the right of the crossover which gave rise to the *H-2^{hc}* chromosome and thus to the right of *Ss-Slp*.

By analyzing the frequency of CRL in 2-wk old congenic mice bearing chromosomes of *H-2-Tla* recombinant type, it was possible to find a right hand limit for *CRL-1*. Thus, CRL frequency in 2-wk old C57BL/6 mice is low. These mice are *H-2^b* and *Tla^b*. A congenic strain exists in which the *Tla^a* gene, derived from an A/J mouse (an intermediate CRL strain), has been placed on a C57BL/6 genetic background. Thus, the *H-2* region of this mouse derives

TABLE III
CRL Frequency in 2-wk Old Mice with Recombinant H-2 Regions

Strains	H-2 complex						CRL frequency %
	Symbol	K	Ir-1	Ir-IgG	Ss-Slp	D	
Parental							
B10.A	a	k	k	k	d	d	14.5 ± 0.8
B10	b	b	b	b	b	b	7.6 ± 0.5
Recombinant							
B10.A (4R)	hd	k	k /	b	b	b	6.1 ± 0.3
B10.A (5R)	ic	b	b /	b /	d	d	14.8 ± 1.2
B10.A (2R)	hc	k	k	k	d /	b	7.4 ± 1.0

CRL frequencies were measured in 14- to 18-day old mice of the strains noted. The genotypes of the mice are indicated by the alleles they possess at the *K*, *Ir-1*, *Ir-IgG*, *Ss-Slp*, and *D* loci. In strains which derive their *H-2* region from recombination, the postulated site of recombination is shown by a slash. 3-21 individual animals of each strain were tested. The results are reported as mean ± standard error. The frequency of CRL in 2-wk old B10.A mice is significantly greater ($P < 0.001$) than the frequency in 2-wk old B10 mice. The frequency of CRL in 2-wk old B10.A(5R) mice is significantly greater ($P < 0.001$) than the frequency in either B10.A(4R) or B10.A(2R) mice.

from a low CRL strain while the *Tla* gene is obtained from intermediate CRL strain. These mice demonstrate a low CRL frequency, suggesting that *CRL-1* is to the left of *Tla* (Table IV). Similarly, the CRL frequency in 2-wk old A/J and A/*Tla*^b mice is the same (intermediate), despite the fact that *Tla* gene (*Tla*^a) of the intermediate CRL CRL A/J strain has been replaced with the allele (*Tla*^b) present in the low CRL C57BL/6 strain. This confirms the location of *CRL-1* as to the right of *Ss-Slp* and to the left of *Tla*. We are at present unable to determine whether *CRL-1* is to the left, within, or to the right of the *H-2D* region (Fig. 1).

Lack of Association of CRL Frequency in 2-wk Old Mice with Ig Allotype.— In order to investigate the possibility of a genetic linkage between the second gene controlling CRL frequency in young mice and the gene complex controlling Ig heavy (H) chains (*IgC_H* region), we determined CRL frequency and Ig allotype of progeny of AKD2 × DBA/2 matings. Mice were splenectomized at 2 wk of age and the CRL frequency determined. At 6 weeks of age, when maternal Ig is no longer detectable in their serum, the mice were bled and the serum tested for the presence of the G⁴H⁴ allotype. This allotype is shared by IgG (γ_{2a}) and IgH (γ_{2b}) molecules of mice possessing the a⁴ *IgC_H* linkage group. AKR mice possess the a⁴ linkage group while DBA/2 mice do not. Hence, the presence of the G⁴H⁴ allotype in the serum of an individual mouse derived from an AKD2 × DBA/2 cross indicates inheritance of the *IgC_H* region of AKR type from the AKD2 parent.

TABLE IV
CRL Frequency of 2-wk Old H-2-TLa Recombinant Mice

Strain	H-2-Tla regions				CRL frequency
	K	Ss-Slp	D	Tla	
B6/TL ⁻	b	b	b	b	4.9 ± 0.2
B6/TL ⁺	b	b	b	/ a	4.5 ± 0.7
A/TL ⁺	k	d	d	a	11.8 ± 0.6
A/TL ⁻	k	d	d	/ b	14.4 ± 2.4

CRL frequencies were measured in 14- to 18-day old mice of the strains noted. The genotypes of the mice are indicated by the alleles they possess at the *K*, *Ss-Slp*, *D*, and *Tla* loci. In strains derived from *H-2-TLa* recombinations, the postulated site of recombination is shown by a slash. Six individual animals of each strain were tested. The results are reported as mean ± standard error. Mean CRL frequency of B6/TL⁻ and B6/TL⁺ are not significantly different ($P > 0.6$) nor is the CRL frequency of A/TL⁺ and A/TL⁻ ($P > 0.3$). CRL frequency of B6/TL⁻ is significantly lower than that of A/TL⁻ ($P < 0.005$). CRL frequency of A/TL⁺ is significantly greater than that of B6/TL⁺ ($P < 0.001$).

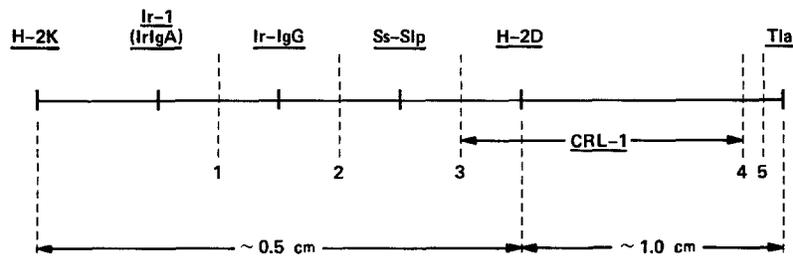


FIG. 1. Schematic map of the *H-2-Tla* region showing locations of *K*, *Ir-1*, *Ir-IgG*, *Ss-Slp*, *D*, and *Tla*. The postulated sites of the recombination events generating the *H-2* regions used in this study are also indicated as is the region in which *CRL-1* appears to be located. Postulated crossover site giving rise to: (1) B10.A(4R); (2) B10.A(5R); (3) B10.A(2R); and (4) C57BL/6TL⁺ and (5) A/TL⁻. The abbreviation cm refers to centimorgans.

Two mice of low CRL type occurred in an AKD2 × DBA/2 backcross litter. Both of these mice possessed Ig with the G⁴H⁴ allotype, indicating the absence of linkage between AKR genes which specify high CRL frequency at 2 wk of age, and the *IgC_H* region (Table V).

DISCUSSION

In this series of papers, we have described several aspects of the appearance and development of B lymphocytes in neonatal mice. In particular, we demonstrated that lymphocytes bearing surface Ig could be identified considerably earlier in life than lymphocytes bearing both surface Ig and receptors for activated C3. Whether this represents a maturational sequence of a single line of

TABLE V
Lack of Linkage of Frequency of CRL in 2-wk Old Mice to Ig_{C_H} Locus

Progeny of (AKD2 × DBA/2) backcross	CRL frequency at 2 wk %	Presence of G ⁴ H ⁴ specificity
1	17.1	+
2	10.2	-
3	9.8	-
4	18.7	+
5	4.5	+
6	5.0	+
7	12.0	-

Backcross progeny of an AKD2 × DBA/2 mating were splenectomized at 2 wk of age and CRL frequency determined. Animals 5 and 6 were classified as low CRL type (<6.6%). At 6 wk of age, the animals were bled and the presence of the G⁴ H⁴ specificity determined. This allotype is characteristic of the A⁴ linkage group of which AKR is a member and thus its presence indicates that the Ig_{C_H} region is derived from the AKR chromosome of the AKD2 parent.

B lymphocytes or, alternatively, disparate rates of development of independent B-lymphocyte lines is unknown. However, we demonstrated that the rate of appearance of lymphocytes bearing C receptors was under genetic control. A strain which had large numbers of CRL at 2 wk of age (AKR) appeared to differ from a strain having few CRL at 2 wk of age (DBA/2) at two independent genetic loci regulating the rate of appearance of CRL.

In this communication, we have identified one of these "CRL genes" (*CRL-1*) and linked it to the *H-2* complex. The evidence offered to support this contention is, firstly, that all low CRL progeny of AKD2 × DBA/2 matings lack the histocompatibility type of the AKR (*H-2^k*). Secondly, the CRL frequency of congenic mice of two different series (the B10 and the A/WySn) is dependent on the *H-2* region they possess. In the study of the congenic mice, it became apparent that the presence of *CRL-1* in a strain that would have otherwise been of low CRL type conveyed to that strain an intermediate CRL frequency (12–15%) as opposed to the low CRL frequency of most strains (7% or less) and to the high CRL frequency of AKR mice (27%). This indicates that the presence of the high allele of *CRL-1* in the absence of the other major CRL gene leads to an intermediate CRL frequency. A more precise localization of *CRL-1* was provided by the measurement of CRL frequency in congenic mice possessing *H-2-T1a* regions derived by recombination between *H-2-T1a* chromosome regions bearing known *CRL-1* alleles. Thus, analysis of congenic B10 mice bearing *H-2* regions derived by recombination between *H-2^a* and *H-2^b* types indicate that *CRL-1* is to the right of the genes for *Ss-Slp*. Similarly, mice bearing *H-2-T1a* regions derived from recombination between *H-2^b*-

Tla^b and *H-2^a-Tla^a* chromosomes have CRL frequencies suggesting a localization of *CRL-1* to the left of *Tla*.

There are several aspects of these findings which bear comment. Firstly, the presence of the high allele of *CRL-1* in inbred strains is not determinable simply on the basis of the *H-2* types of these animals. For example, AKR mice, which type serologically as *H-2^k*, possess the high allele of *CRL-1* (as well as the high allele of the other, unidentified CRL gene) while C57BR, B10.BR, and C3H mice, which also type serologically as *H-2^k*, lack the high allele of *CRL-1*. This is consistent with a position of *CRL-1* outside of *H-2* itself, although within the *H-2-Tla* region. On the other hand, the similarity of *H-2* regions of mice of distinct origin based upon typing for the known *H-2* region products (*K*, *Ir-1*, *Ir-IgG*, *Ss-Slp*, and *D*) does not establish that all the genes of their *H-2* regions are identical. Thus, this observation does not rule out a location of *CRL-1* within *H-2* itself. Similarly, possession of an *H-2D* region of similar type to that of a strain bearing the high allele of *CRL-1* does not insure the presence of high *CRL-1* allele. For example, BALB/c, DBA/2, and B10.D2 mice are all *H-2^d* and bear the *d* allele of the *H-2D* gene as does the A strain. Nonetheless each of these *H-2^d* strains lack the high allele of *CRL-1* while A mice possess this allele. Similarly, AL/N mice which have an *H-2* region of distinct origin from, although similar in many respects to, that of A mice lack the high *CRL-1* allele. This again suggests either a lack of identity of all genes in the *D* region of these mice or indicates that *CRL-1* is to the right of *H-2D*, although to the left of *Tla*.

Secondly, the precise process which *CRL-1* controls remains unknown. It does not appear to be a structural gene for the C receptor as it controls the time of appearance of cells bearing these receptors rather than their presence or absence. The gene likely plays some role in the differentiation of the B-lymphocyte line but the nature of the function of its product remains obscure. Indeed, the crucial question as to whether *Ig⁺*, C receptor⁻ and *Ig⁺*, C receptor⁺ lymphocytes are forms of a single line of B lymphocytes or representatives of individual lines has not yet been resolved.

The location of *CRL-1* in the *H-2* region is most interesting in view of the mounting evidence that many functions relating to the immune system are controlled in this region. Thus, it is now recognized that genes controlling immune responses to individual antigens (*Ir* genes) (10, 11), mixed lymphocyte responses (*MLR* genes) (12), antigens mainly expressed on lymphocytes (*Ir1.1*, *Lna*, β) (13-16), the thymus leukemia antigen (*Tla*) (9), serum complement levels (*Ss-Slp*) (17), and major histocompatibility antigens (*K* and *D*) (7, 8) are all located in the *H-2-Tla* complex. Moreover, it has recently been shown that efficient interaction between immunocompetent cells in guinea pig and mouse systems depends upon similarity in the major histocompatibility complex (MHC) (18, 19) and either a sharing of a particular *Ir* gene product or of a possible MHC controlled interaction structure.

To our knowledge *CRL-1* is the first gene controlling B-cell differentiation which has been localized to this region, although it has been reported that genes controlling surface antigens preferentially expressed on B lymphocytes are in the *H-2* region (16). The latter genes are more closely linked to the *K* region than to the *D* region and may be near (or equivalent to) *Ir* genes. *CRL-1*, on the other hand, is on the *D* side of *H-2* or to the right of *D*.

We have made one unsuccessful attempt to localize the other gene involved in control of CRL frequency in 2-wk old mice. We found no relation between Ig allotype and CRL frequency in 2-wk old progeny of AKD/2 \times DBA/2 mice. This suggests that this other CRL gene is not in the *IgC_H* region.

Finally, one other gene important in B-lymphocyte function has recently been localized to the X chromosome in mice. Thus, CBA/HN mice make little or no antibody response to several thymus-independent antigens. Genetic studies demonstrate that the "defective" gene in these animals is X linked (20). We have not yet established whether the non-*H-2*-linked CRL gene is X linked.

A more complete genetic and functional analysis of the CRL genes described in this and the preceding paper promises to yield significant insight into normal and abnormal control of lymphocyte differentiation.

SUMMARY

The frequency of lymphocytes bearing complement receptors in the spleens of 2-wk old mice appears to be controlled by two independent genes. The presence of a "high" allele at either locus leads to intermediate or high frequency of CRL at 2 wk of age. One of the genes controlling complement receptor lymphocyte (CRL) frequency (*CRL-1*) is linked to the *H-2* complex. Thus, in progeny of (AKR \times DBA/2) F_1 \times DBA/2, all mice with a low frequency of CRL at 2 wk of age are homozygous for the *H-2* type of the low CRL parent (DBA/2). Furthermore, in the B10 series of congenic mice, CRL frequency at 2 wk of age is similar to the frequency in the donor of the *H-2* region. Thus, C57BL/10, B10.BR, and B10-D2 mice are all of the low CRL type while B10.A mice are intermediate in CRL frequency at 2 wk. C57BR and DBA/2, the donors of the *H-2* complex of the B10.BR and B10.D2, respectively, are of low CRL type while the A/WySn, the donor of the *H-2* complex in the B10.A, is an intermediate CRL strain. Similarly in the A/WySn series of congenic mice, A.CA, A.SW, and A.BY are all low CRL strains while the A/WySn is intermediate.

Studies of CRL frequency in mice with recombinant *H-2* chromosomes (B10.A(2R), (4R), and (5R); B6/TL⁺; and A/TL⁻) indicate that *CRL-1* is to the right of the *Ss-Slp* genes and to the left of *Tla*.

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