Brief Definitive Report

DETERMINATION OF THE KAPPA ANTI-α(1,3) DEXTRAN IMMUNE RESPONSE DIFFERENCE BY A GENE(S) IN THE V_κ-LOCUS OF MICE*

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A single injection of dextran B1355 into BALB/c mice results in a rapid λ_1 anti-α(1,3) dextran response (1–3). One of the genes controlling this response is in the heavy chain locus (1, 4) and is symbolized as $v_{H\lambda 1}^{\alpha(1,3)}$ (3). Mice lacking this gene fall into two classes: those that yield a κ-anti-α(1,3) response after repeated injections and those that never respond. This latter response difference appears to be due to a $v_\kappa^{\alpha(1,3)}$-gene which is the subject of this paper.

Edelman and Gottlieb (5) were the first to report the simple Mendelian inheritance of a V_κ framework peptide I_B. By using the I_B marker, Gottlieb (6, 7) mapped the v_κ-locus close to Ly-3 which codes for a T cell-specific alloantigen. Hengartner et al. (8) then showed the v_κ-locus to be present on chromosome 6 which carries the Ly-3 locus also. Polymorphism in v_κ-genes has been analyzed by (*a*) isoelectric focusing of κ-chains in normal serum immunoglobulin (9); (*b*) isoelectric focusing of κ-chains in antibodies to phosphorylcholine (10, 11); and (*c*) the inheritance of an idiotype present on antibodies to phenylarsonate (12). In all four experimental systems (5–12), it was found that the inbred strains AKR/J, C58/J, PL/J, and RF/J were distinct from all other mice in that they expressed an allogroup designated v_κ^a which is always associated with the Ly-3.1 allele (7, 13).

Here we extend the above findings (5–12) by showing that an all-or-none κ-anti-α(1,3) immune response difference distinguishes the v_κ-loci of C57BL/6 and BALB/c mice, both of which express the Ly-3.2 allele. Consequently, three v_κ-allogroups, a, b, and c, are defined, the prototypes of which are carried, respectively, by the strains AKR, C57BL/6, and BALB/c.

Materials and Methods

Mice. C57BL/6J (The Jackson Laboratory, Bar Harbor, Maine); CB20 (M. Potter), CB20B (B. Blomberg), AKR/N mice (D. Nebert), recombinant inbred strains, CXB, derived from BALB/c and C57BL/6 (D. Bailey), beta 16 and M16 (E. Boyse), and B6-PL/Cy (M. Cherry) were maintained by our own breeding colony. CE/J were purchased from The Jackson Laboratory. The markers expressed by these mice are summarized in Table II.

Dextran Antigens. Dextran purified from the capsular polysaccharide of *Leuconostoc mesenteroides* was a gift from Dr. A. Jeanes (U. S. Department of Agriculture). Dextran B1355 fraction

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S is a glucopyranoside polymer containing 57% α(1,6) and about 43% α(1,3) linkages (14).[1] B512 is a glucopyranoside polymer with 95% α(1,6) linkages and devoid of α(1,3) linkages.

Immunizations. Weekly, mice were injected intraperitoneally with 0.1 mg dextran B1355 dissolved in phosphate-buffered saline and bled 7 d after each injection. Mice 6–8 wk of age were started on this protocol.

Purification of Specific Antibodies. Anti-λ_1, anti-κ, and anti-α(1,3) dextran were purified by affinity chromatography as described or referenced previously (3).

Immunoadsorbant Columns. Proteins were covalently attached to a CNBr-activated Sepharose 4B (Pharmacia Fine Chemicals, Piscataway, N.J.) chromatographic support and antibodies eluted as described or referenced previously (3). Similarly, polyacrylamide dextran B1355 or dextran B512 gels were prepared as described (3).

Iodination of Proteins. Anti-κ or anti-λ_1 antibodies were iodinated by a chloramine-T oxidation method (3).

Radioimmunoassay. The λ_1 or κ-antibodies to α(1,6) or α(1,3) glycosyl linkages were quantitated by a solid phase radioimmunoassay (3).

Isoelectric Focusing Patterns of Anti-α(1,3) Dextran κ-Chains. The κ-chain heterogeneity was analyzed according to Gibson (9).

Results

The specificity of the response was analyzed by comparing the binding of antibody to dextran B1355 containing both α(1,3) and α(1,6) glycosyl linkages, with the binding to dextran B512 containing only the α(1,6) linkage. In addition, the class of L chains (κ vs. λ) of these antibodies was determined. As shown in Table I, the amount of λ_1-bearing antibodies is low or nil (the background of the assay is $\cong$10) in mice lacking the $v_{H\lambda 1}^{\alpha(1,3)}$-gene. However, they all respond to α(1,6) glucosyl linkages, although the maximum of the response is reached only after four or more injections. In contrast, there clearly are two categories of κ-responses to the α(1,3) determinant: some strains mount a κ-anti-α(1,3) response equivalent to their κ-anti-α(1,6) response; other strains, even after hyperimmunization, hardly respond. No response difference was found between males and females, either in a "responder" (C57BL/6) or "nonresponder" (CXBK) strain.

C57BL/6, CXBE, and CXBI gave quantitatively similar responses. The absence of an anti-α(1,3) response in CXBK is due to a BALB/c allele not in H-2 or the heavy chain allogroup because CB20B mice (BALB/c, H-2^b, Ig^b) are nonresponders (Table I).

The F_1 offspring from two combinations of responder by nonresponder parents are responders; and no complementation was observed in F_1 offspring from two combinations of nonresponder strains (Table I).

The anti-α(1,3) κ-chains would be expected to display restricted heterogeneity because only one or a few v-genes could be responsible for the response difference. To analyze this, the κ-chains from anti-α(1,3) antibodies of individuals were run on an isoelectric focusing gel. The pattern indicates a limited heterogeneity of κ-chains which is similar in C57BL/6J, CXBE, and CXBI mice (Fig. 1). This result is in agreement with the hypothesis that the v_κ-locus determines the response difference.

To map this locus, the response to dextran B1355 was analyzed in four strains of C57BL/6 mice congenic except for chromosome 6 which carries the Ly-3 locus. Table I shows that C57BL/6 and Beta 16 (B6, Ly-3.2) are "responders," whereas M16 and B6-PL/Cy (B6, Ly-3.1) are "nonresponders." Given a C57BL/6 background, this links the gene(s) determining the response difference to the Ly-3 locus (see Table II).

[1] Jeanes, A. Personal communication.

TABLE I

Humoral Response to Dextran B1355

Strain	Immunization	Antidextran antibodies*			
		α(1,3) Specificity		α(1,6) Specificity	
		κ-Class	λ_1-Class	κ-Class	λ_1-Class
			μg/ml		
C57BL/6J	1°‡	0	13	112	11
	2°‡	40	10	160	10
	HI‡	244	30	278	14
CXBE	1°	52	17	47	9
	HI	264	22	125	9
CXBI	1°	30	6	43	6
	HI	120	8	134	20
Beta 16	1°	56	13	80	11
	HI	150	35	130	23
CB20	1°	0	0	112	12
	HI	14	7	130	17
CB20B§	1°	0	1	120	10
	HI	14	7	240	12
CXBK	1°	10	0	25	4
	HI	19	7	198	9
M16	1°	0	3	41	2
	HI	12	5	152	12
B6-PL/Cy	1°	8	ND‖	168	ND
	HI	7	ND	213	ND
AKR/N	1°	4	ND	142	ND
	HI	8	ND	202	ND
CE/J	HI	10	ND	85	ND
(CXBE × CXBK)F_1	1°	150	ND	95	ND
	HI	280	ND	125	ND
(Beta 16 × M16)F_1	1°	0	ND	130	ND
	HI	120	ND	230	ND
(M16 × CB20B)F_1	1°	0	ND	78	ND
	HI	0	ND	135	ND
(M16 × CXBK)F_1	HI	3	ND	220	ND

* Pooled sera from 5–20 mice were assayed by radioimmunoassay. These values (micrograms per milliliter) do not represent the absolute quantity of antibody binding to dextran in test samples, but rather that amount equivalent to a standard myeloma protein which binds the same quantity of the indicated (anti-λ_1 or anti-κ) probe.

‡ 1^0, 2^0, primary, secondary response; HI, hyperimmune (5–10 injections).

§ In our previous study (15), no distinction was made between the responses κ-anti-α(1,3) and anti-α(1,6) when immunizing with dextran B1355. The CB20B response reported there (15) is due to κ-anti-α(1,6) and is not in contradiction with the finding reported here that CB20B is a nonresponder in the κ-anti-α(1,3) class.

‖ Not done.

Conclusion

Mice of the Ig^b heavy chain allogroup cannot respond to produce λ_1 anti-α(1,3) dextran because they lack the $v_{H\lambda 1}^{\alpha(1,3)}$-gene. If a strong immunogenic selection pressure is now placed on them by hyperimmunization with dextran B1355, they respond as follows: All strains produce similar amounts of antibody in the κ-class to α(1,6) linkages. However, after repeated injections, "responders" synthesize κ-anti-α(1,3) dextran at a level comparable to the κ-anti-α(1,6) response, whereas "nonresponders" synthesize κ-anti-α(1,3) dextran at a level at least 10-fold below that of the κ-anti-α(1,6) response generally close to the background of the assay (Table I).

The κ-anti-α(1,3) dextran shows a restricted pattern of heterogeneity (Fig. 1) essentially identical in the three responder strains (C57BL/6J, CXBE, CXBI). This in itself suggests a role of structural v_κ-genes in determining the response difference.

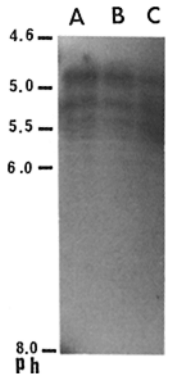

FIG. 1. Isoelectric focusing pattern of κ-chains from purified anti-α(1,3) dextran antibodies. (A) C57BL/6; (B) CXBE; (C) CXBI. Dark bands focusing between pH 4.8 and 5.5 appeared on the film after 2 d of exposure. Faint bands focusing between pH 5.5 and 6.0 appeared only after 8 d of exposure.

Genetic studies confirm this hypothesis. Inasmuch as the response difference occurs within strains possessing the H-2^b haplotype and the Ig^b allogroup ("responder" C57BL/6, CXBE, CXBI, Beta 16 vs. "nonresponder" CXBK, M16, B6-PL/Cy), another locus from the parental strains BALB/c and C57BL/6 unlinked to H-2 and $Ig,^b$ must determine the production of κ-anti-α(1,3) antibodies. In fact, the difference due to this locus becomes visible by simply comparing the positive response of C57BL/6 mice with the negative response of CB20B mice (Table II). This leads to the conclusion that the "responder" CXBE and CXBI lines carry the immune response gene(s) of C57BL/6, whereas the "nonresponder" CXBK carries the gene(s) of BALB/c.

The response of four congenic strains of C57BL/6 mice ("responder" C57BL/6, Beta 16 vs. "nonresponder" M16, B6-PL/Cy) maps the immune response locus in the XI^{th} linkage group (chromosome 6) very close to Ly-3. Given the demonstration that the v_κ-locus is linked to Ly-3 (6, 7) and on chromosome 6 (8), we are in all likelihood mapping the structural gene $v_\kappa^{\alpha(1,3)}$ (see Summary and Table III). Mice carrying the Ly-3.1 allele (M16, B6-PL/Cy, AKR/N) are "nonresponders," i.e., they are $v_\kappa^{\alpha(1,3)^-}$. Mice carrying the Ly-3.2 allele are all I_B^- and fall into both "responder" and "nonresponder" classes. Thus, three v_κ-allogroups are revealed as shown in Table IV.

As would be expected of a structural v_κ-gene, responsiveness is dominant (Table I). At the moment, only one germ line $v_\kappa^{\alpha(1,3)}$-gene shared by all "responder" strains need be assumed. The fact that we see a similar and restricted IEF pattern for the κ-anti-α(1,3) dextran in the various "responder" strains (Fig. 1) supports this.

Given the known behavior of the germ line $v_{H\lambda 1}^{\alpha(1,3)}$-gene which permits a rapid high response in the λ_1-class, it is surprising that the germ line $v_\kappa^{\alpha(1,3)}$-gene is maximally expressed only after weeks of hyperimmunization.

We suggest the following (albeit not unique) interpretation of this slow response: The germ line $v_\kappa^{\alpha(1,3)}$-gene complements with a $v_{H\kappa}^{\alpha(1,3)}$-gene in the Ig^b allogroup to produce an antigen-recognizing receptor of marginal affinity for the α(1,3) determinant. On long-term immunization, somatic mutants of higher affinity are selected. The difference between a responder and nonresponder is in the number of mutational steps required to achieve a κ-anti-α(1,3) affinity high enough to compete in inducibility with κ-anti-α(1,6). The IEF pattern (Fig. 1) would not distinguish these mutants if, within a given framework encoded by $v_\kappa^{\alpha(1,3)}$, the complementarity-determining replacements were neutral or buried in the heterogeneity as a result of deamination in serum of the κ-chain.

TABLE II

Assignment of the $v_\kappa^{\alpha(1,3)}$-Allele

Strain	Ig-1	H-2	Ly-2	Ly-3	v_κ*	I_B‡	$v_\kappa^{\alpha(1,3)}$§
C57BL/6	b	b	2	2	B	−	+
CXBE	b	b	2	2	(B)	(−)	+
CXBI	b	b	2	2	(B)	(−)	+
CB20	b	d	2	2	C	(−)	−
CB20B	b	b	2	2	C	(−)	−
CXBK	b	b	2	2	(C)	(−)	−
Beta 16‖	b	b	1	2	C3H/An (or B)	−	+
M16	b	b	1 RF	1 RF	(RF)	+	−
B6-PL/Cy	b	b	1 PL	1 PL	(PL)	+	−
AKR/N	d	k	1	1	AKR	+	−
CE/J	f	k	1	2	CE	(−)	−
(CXBE × CXBK)F_1	b	b	2/2	2/2	(B/C)	(−)	+
(Beta 16 × M16)F_1	b	b	1/1	2/1	(B/RF)	(+)	+
(CB20B × M16)F_1	b	b	2/1	2/1	(C/RF)	(+)	−
(CXBK × M16)F_1	b	b	2/1	2/1	(C/RF)	(+)	−

* Strain of origin of the v-allogroup. Parentheses are used when the origin is inferred from κ-anti-α(1.3) response of prototype strains: C, BALB/c, B, C57BL/6.

‡ V_κ framework peptide marker (5–7). Parentheses are used when the phenotype is not experimentally known.

§ κ-Response to α(1,3) glucosyl linkages taken from Table I.

‖ Because Beta 16 derived its chromosome 6 from C3H/An, which possesses the same Ly-3.2 allele as C57BL/6, it is not possible to decide whether the responder phenotype is due to a crossover with C57BL/6 between Ly-2 and Ly-3 or whether C3H/An itself expresses the $v_\kappa^{\alpha(1,3)+}$ and I_B^- alleles.

TABLE III

Known Markers of the v_κ-Locus

Author methodology	v_κ^a	v_κ^b	References
Fingerprint of pooled normal κ-chains	I_B^+ Ly-3.1 AKR/J, C58/J, RF/J, PL/J, C57BL/6-Ly-2.1-Ly-3.1 AKR.B6/1	I_B^- Ly-3.2 DBA/1J, SWR/J, ST/bJ, CBA/2J, C57L/J, BDP/J, A/J, CBA/J, 129/J, A/HeJ, C57BL/6J, MA/J, C3H/HeJ, C57BL/KsJ, SEA/GnJ, NZB, AL/N	5–7
IEF patterns of κ-chain from purified PC8 Id^+ antiphosphorylcholine	Pattern κ-PC8-A AKR/J, C58/J, RF/J, PL/J (AKR/J × C57L/J)F_1 (AKR/J × DBA/2J)F_1	Pattern κ-PC8-B C57L/J, BALB/cJ, CBA/J, C3H/HeJ, MA/MyJ, ST/6J, 129/J, SEC/ReJ, C57BL/6J, DBA/2J, AL/N, A/J, CE/J	10, 11
IEF patterns of pooled normal κ-chains	AKR/J, C58/J, RF/J	SWR/J, C3H/HeJ, DBA/1J, A/J, C57BL/6J	9
Segregation of A/J Id of anti-ARS	v_κ Id^- PL/J	v_κ Id^+ A/J	12

TABLE IV

The Three Allogroups of the Murine v_κ-Locus

	v_κ^a	v_κ^b	v_κ^c	Reference
Prototype strain	AKR	C57BL/6	BALB/c	
Ly-3 allele	Ly-3.1	Ly-3.2	Ly-3.2	13
I_B peptide	I_B^+	I_B^-	I_B^-	5–7
κ-Anti-α(1,3) dextran response	Nonresponder ($v_\kappa^{\alpha(1,3)-}$)	Responder ($v_\kappa^{\alpha(1,3)+}$)	Nonresponder ($v_\kappa^{\alpha(1,3)-}$)	This paper

Summary

Mice lacking the $v_{H\lambda 1}^{\alpha(1,3)}$-gene do not produce a λ_1 anti-α(1,3) dextran response. However, on hyperimmunization some strains mount a κ-anti-α(1,3) dextran response, whereas others remain nonresponder. Responsiveness is dominant.

The κ-anti-α(1,3) response difference is linked to the Ly-3 locus on chromosome 6 and is likely the result of a structural v_κ-gene(s). In conjunction with previous work, three v_κ-allogroups can now be distinguished. At present, this is the only example of an immune responsiveness difference associated with the v_κ-locus.

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