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Immunology

Mice completely suppressed for the expression of immunoglobulin κ light chain

(immunoglobulin λ light chain/compensation of κ chain loss/heterogeneity/immunological repertoire)

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ABSTRACT **Complete suppression of expression of immunoglobulin κ light chain was achieved by injecting female mice from birth with a mixture of antisera against the μ heavy chain and κ light chain (anti-μ and anti-κ). Then their offspring were injected with anti-κ from birth. This resulted in stable suppression as long as anti-κ injections were continued. κ light chain was not detectable either in serum or at the cellular level. The number of B cells in spleen and the concentration of immunoglobulin classes and subclasses in serum were normal. The normal levels were achieved by a compensating enhancement of λ light chain expression. Analysis of the light chains of immunoglobulins secreted by spleen cells from suppressed mice after liposaccharide stimulation by two-dimensional gels showed λ chain to have a limited heterogeneity. Primary responses to dinitrophenol, influenza strain A, and keyhole limpet hemocyanin were drastically affected, whereas secondary responses appeared to be quite normal, suggesting a surprisingly large potential repertoire.**

Two types of immunoglobulin light chains, κ and λ, are present in mammals and birds (1, 2). The reason for the parallel existence of two light chain types is unclear, especially because some species show a remarkable imbalance towards one or the other. In the horse, for example, λ light chains are carried by about 95% of serum immunoglobulins (2), whereas in the mouse λ light chains constitute only 2–5% of the serum light chain pool (2, 3). From sequence data of mouse myeloma proteins, it was concluded that this imbalance is a reflection of the number of germ-line genes for the variable (*V*) part of the particular light chain (4). Indeed, current estimates on the number of germ-line genes based on analysis at the DNA level are 100–200 *V* genes for κ (V_κ) and two for λ (V_λ) (5–7).

The relative contribution of the two classes of light chain to the immune response can be evaluated in mice that express only one type of light chain. The loss of λ chain seems to have no drastic effects because SJL and BSVS mice, which display a lower expression—$^1/_{50}$th—of λ chain than do other mouse strains (3, 8, 9), are not noticeably immune deficient.

The effect of a loss of expression of κ chain should be more drastic, given its major contribution (>90%) to the normal repertoire. The immune deficit due to loss of expression of κ chain would be expected to depend upon the number of germ-line V_λ genes that the animal expresses. In the rabbit (normal level of κ chain, 90–95%; refs. 2, 10), a strain exists that has lost the expression of the major κ-type chain and whose immune system behaves quite normally (10). In this case it was shown that the V region of λ chain is encoded by multiple (>20) germ-line genes (11), and a second κ chain locus is expressed in higher quantities than normal (12). Because in mice only two V_λ genes exist, the complete loss of the expression of κ chain should result in visible holes in the repertoire.

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MATERIALS AND METHODS

Mice. Balb/c mice were obtained from the animal colony of the Salk Institute.

Proteins and Antibodies. The purification of proteins and antibodies and their modification and use have been described in detail (13–16).

Methods. Radioimmunoassays, two-dimensional gel electrophoresis, haptenated phage inactivation, and passive hemagglutination were done exactly as described (13, 17–20).

RESULTS

Establishment of Suppression. In order to suppress the expression of κ light chains in mice (κ suppressed mice) female parents were treated with antiserum to μ heavy chain (anti-μ) to render the animals free of immunoglobulins. Then, the offspring of these mothers were treated with antiserum to κ light chain (anti-κ). With this schedule, two attempts to obtain κ suppressed mice were unsuccessful, probably because of the presence of κ chain-bearing IgG in the parental suppressed females (21, 22). In a third attempt, the parental females were injected daily with anti-IgM (anti-μ and anti-κ) intraperitoneally starting with 200–400 μg from birth through week 1 and then with increasing doses twice a week, reaching finally 1 mg per injection at age 1 month. Starting with the time of mating, only anti-κ (1 mg per injection) was given. The two females used for breeding had little but measurable κ chain immunoglobulins in their sera (10^{-4} of normal).

The offspring of these mice were injected with anti-κ by the schedule described for anti-IgM suppression. In the first litter of 11 babies, 1 died after 2 days and the rest survived. They were bled at age 2 months from the tail artery (eye-bleeding resulted in severe infections), and their sera were tested for the presence of κ and λ light chains by the radiobinding assay. None of them contained any detectable κ light chains, and the λ chain concentration was comparable to that in sera from normal mice. However, at age 3–4 months, still no κ light chains were detectable in these mice (assayed to the level of <10 ng/ml of IgM and 100 ng/ml of IgG), but the λ chain concentration had increased ≈30-fold (Fig. 1). This 30-fold increase of λ chain was observed in all other κ

Abbreviations: DNP, dinitrophenol; KLH, keyhole limpet hemocyanin; CGG, chicken gamma globulin; LPS, lipopolysaccharide; anti-λ, anti-κ, and anti-μ, antisera against the immunoglobulin λ light chain, κ light chain, and μ heavy chain; V, variable; V_κ and V_λ, V region genes for κ and λ light chains; κ suppressed mice, mice in which the expression of κ light chain is suppressed.

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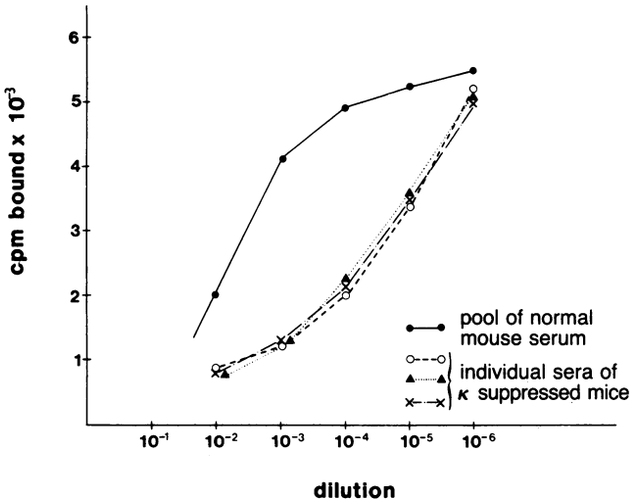

FIG. 1. Inhibition of binding of anti-λ to J558 by normal mouse serum and by three individual sera from κ suppressed mice. All other κ suppressed mice gave similar results.

suppressed mice tested thus far. The low total immunoglobulin at ≤2 months was most likely due to residual anti-μ antibodies transferred from the mother, because it was never observed in suppressed mice born to mothers that were also κ suppressed and had never received anti-μ antibodies. Semiquantitative estimation of the concentration of immunoglobulin classes and subclasses (21) revealed similar titers in sera from κ suppressed and normal mice. This means that the lack of expression of κ chain is compensated by an increased level of λ chain immunoglobulins.

The suppression could be maintained easily by injecting the mice with 1 mg of anti-κ twice a week. The offspring of such female mice also could be suppressed easily. No suppression was observed by injection of the same amount of normal rabbit IgG into babies from suppressed mothers. However, the λ chain level was slightly increased (3-fold) compared to normal mice.

The amount of 1 mg of anti-κ per injection was found necessary for the maintenance of suppression. With 0.5 mg per injection, a patchy type of suppression resulted with only about half the mice remaining suppressed. Less anti-κ or extending the intervals between the injections resulted in the breakage of suppression.

When injections of anti-κ were stopped, κ light chains became detectable in serum after 1.5 months and reached normal levels after ≈4–5 months. However, the elevated levels of λ chain remained almost unaltered. Even 9 months after the first detection of κ chain, when κ chain levels had returned to normal and remained there, the λ chain concentration was still 20- to 30-fold higher than that of normal mice.

The absence of κ chain immunoglobulins in the serum of suppressed mice was reflected also at the cellular level. Anti-κ-treated mice had normal numbers of spleen cells of which about 30–40% were positive for λ chain. No cells positive for κ chain could be found. However, precursors of κ chain-producing cells must have been present because, after stimulation with lipopolysaccharide (LPS), small amounts of κ chain were measurable in the culture supernatant (Fig. 2). This finding was confirmed by cell fusion of LPS blasts from spleen cells of suppressed mice. About 5% of the hybrids secreted κ chain (unpublished data). The bone marrow cells of suppressed mice seemed not to be affected by the anti-κ treatment because after LPS stimulation, the same amount of κ chain-containing immunoglobulin could be detected in culture supernatants from normal and from suppressed mice (Fig. 2). The amount of λ light chain secreted by bone marrow cells from treated mice seemed to be enhanced, but this might be due to contaminating mature B cells in the bone marrow preparation. When κ and λ chains are compared in this figure, λ chain seems to be at an equal or higher level than κ chain. This result is, however, due to the differential sensitivity of the anti-κ and anti-λ (antiserum to λ light chain) reagents.

Diversity of the Immunoglobulins of κ Suppressed Mice. In order to investigate how heterogeneous the immunoglobulins synthesized by suppressed mice are, spleen cells from κ suppressed mice, normal mice, or mice injected with normal rabbit immunoglobulin from birth were stimulated with LPS, and the secreted immunoglobulins were analyzed and compared by two-dimensional gel electrophoresis (Fig. 3). This question is particularly probing because these mice can utilize only two *V* genes for their light chain pool. Spleen cells from animals injected with normal rabbit immunoglobulin (data not shown) displayed similar patterns compared to cells from normal mice.

Several conclusions can be drawn from these gels. Compared to spleen cells from normal mice, cells from κ suppressed mice exhibit a limited heterogeneity regarding their light chains. The two darkest spots were identified as the respective position of proteins encoded by unmutated genes for λ_1 and λ_2 chains (shown by arrows in Fig. 3). It also shows that λ_1 and λ_2 chains are present in comparable concentrations. The spot corresponding to the protein encoded by the unmutated λ_3 gene could not be unambiguously identified, indicating that λ_3 is only a minor component in the light chain pool of these mice.

The nature of the suppression of κ chain expression in these mice can also be evaluated from the light chain patterns of the two-dimensional gels. The presence of only a few minor spots in the suppressed pattern indicates that these are variants of λ chain, residual κ chains, or both. We feel the data overwhelmingly favors the former possibility. First, negligible κ chain was found in this supernatant as detected by the radiobinding assay. Second, the limited heterogeneity of these minor spots compared with that of the normal mouse indicates that, if these are κ chains, only a restricted portion of the κ chain repertoire is expressed, a possibility considered unlikely. Furthermore, most of the minor spots, including the most prevalent one, seem to be in

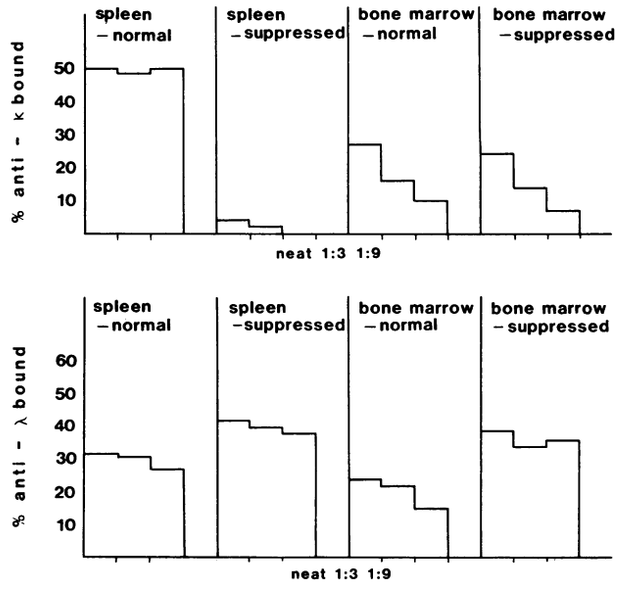

FIG. 2. Radiobinding assay for the κ and λ chain content of the culture supernatants from LPS-stimulated spleen and bone marrow cells from normal and κ suppressed mice.

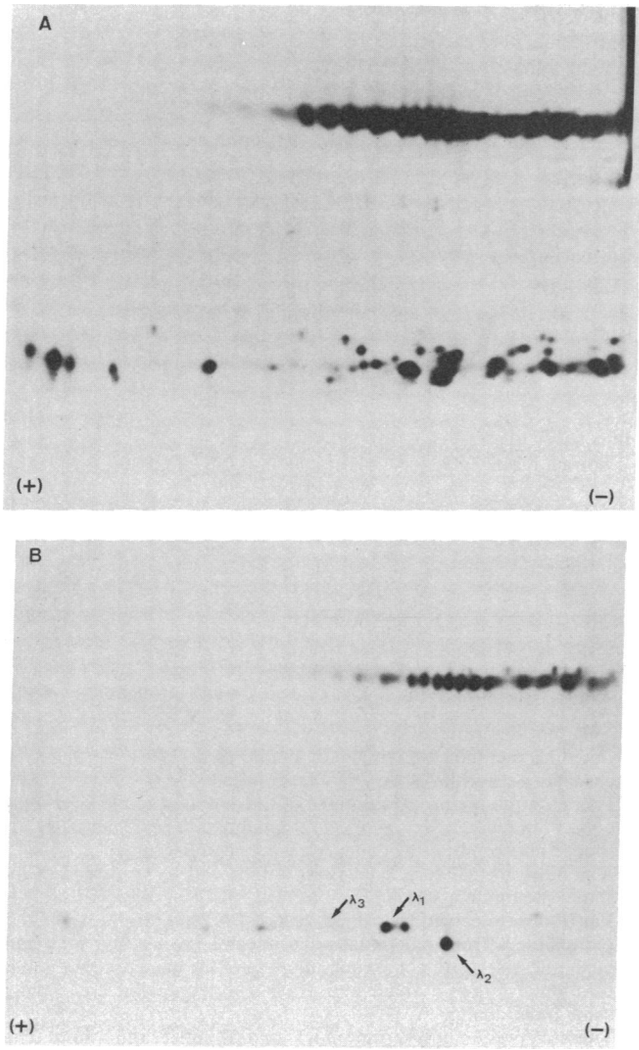

FIG. 3. Two-dimensional gel analysis of secreted immunoglobulins from culture supernatants of LPS-stimulated spleen cells labeled with [^{35}S]methionine from normal (*A*) and κ suppressed mice (*B*). The first dimension of isoelectric focusing (pH range from 5 to 7) was performed in the horizontal direction. The positions of myeloma λ_1, λ_2 and λ_3 chain standards are shown.

one horizontal line regarding electrophoretic mobility in the second dimension, a line that corresponds to the mobility of λ_1 chains. The most reasonable explanation of the data is that most of the minor spots are somatic variants of λ_1 chain, with the substituted amino acids contributing charge differences. Because λ_1 and λ_2 chains are clearly distinguishable by size in our gel system, the data also suggests that λ_2 chain lacks extensive variability. From the comparison of the intensity of the major and minor spots, we can conclude that a large portion of the secreting lymphocytes express genes that still have the germ-line sequence. However, all of the above conclusions have to be tempered by the fact that the isoelectric-focusing first dimension only separates on the basis of charge, and mutant chains with neutral amino acid exchanges will fall in the same position as the germ-line chain. These conclusions are presently being tested in further detail through analysis of hybridomas from κ suppressed mice.

A comparison of heavy chains expressed by normal and suppressed mice by two-dimensional gels is rather difficult because of the multiple spots exhibited even by the heavy chain of a monoclonal immunoglobulin. The pH ranges spanned by the heavy chains of κ and λ chain-bearing immunoglobulins seem to be similar at comparable levels of exposure. (In Fig. 3, the heavy chains from the normal mouse are much more heavily exposed than those of the κ suppressed mouse in order to get equivalent exposure of the light chains in the two patterns.) This was confirmed in gels with wider pH ranges in the first dimension (not shown). However, no statement concerning the heterogeneity can be made with this method of analysis.

The relative amounts of λ_1, λ_2, and λ_3 chains were analyzed also by membrane fluorescence with a polyvalent anti-λ chain antiserum and a monoclonal anti-λ_1 antibody. In young κ suppressed mice (2–4 months old), about 2/3 of the number of λ chain-bearing cells were stained with the anti-λ_1 reagent. This was also confirmed by cell fusion of LPS blasts from such mice (40–50% λ_1-positive hybridomas and about 10% positive for λ_3; unpublished data). In old mice (older than 1 year) the number of λ_1-positive cells was almost equal to the total number of λ chain-bearing cells. Again this could be confirmed by cell fusion of LPS blasts (>90% were λ_1 positive; unpublished data). This interesting phenomenon will have to be explored in more detail.

Immunological Repertoire of κ Suppressed Mice. We have tested only a handful of antigens thus far.

(*i*) With unprimed LPS blasts, only a few cells from κ suppressed mice were found to lyse sheep or horse erythrocytes (10 per 10^6 compared with 10^3 per 10^6 in normal mice).

(*ii*) The anti-dinitrophenol (DNP) response in normal mice is largely in the κ light chain class. A couple of monoclonal antibodies have anti-DNP activity and have the λ light chain (23, 24). The one on which extensive sequence data is available involves a somatic mutant of λ_2 chain that differs by four complementarity-determining amino acids from the germ-line-encoded sequence (25). The experiment in Fig. 4 shows that a large secondary anti-DNP response can be elicited in the λ_1 chain class, suggesting that no or few mutational steps are required to generate this activity from the germline $V_{\lambda_1}V_H$, where V_H = variable region heavy chain genes. These data were confirmed by using anti-DNP-specific hybridomas prepared from κ suppressed mice (unpublished data). The response to DNP in suppressed mice was determined by intraperitoneal immunization with 100 μg of DNP_9-keyhole limpet hemocyanin (KLH; a gift of M. Julius) in complete Freund's adjuvant for primary response and in incomplete Freund's adjuvant for secondary response. Blood was taken 2 weeks after each injection, and the sera were titrated in a phage-inactivation assay. Fig. 4 shows the titration curves of the primary- and secondary-response antisera of individual κ suppressed mice compared with antisera from mice that were injected with normal rabbit IgG from birth. Sera from completely untreated mice displayed similar titration curves compared with sera from mice injected with normal rabbit IgG. The titration curves of the primary response of both types of mice were nicely clustered, and the titer of suppressed mice was one order of magnitude lower than that of control ones. The titration curves of the secondary response of suppressed and control mice were scattered. Based on the limited data, no difference between the two groups could be found. Note the different scales of the inhibition curves of the primary and secondary responses in Fig. 4.

(*iii*) The anti-influenza response is also generally V_κ encoded (26). We studied the effect of immunization with influenza strain A (H3N2). Titration of the antisera showed no activity in the primary response; but in the secondary response of the two κ suppressed mice tested, one had a titer as high as that of normal mice and the other one was almost as high. (These assays were kindly performed by S. Fazekas de St. Groth.)

(*iv*) In the study of the anti-DNP response, two carriers were used, KLH and chicken gamma globulin (CGG). Because the coupling ratios of DNP to carrier were low, one

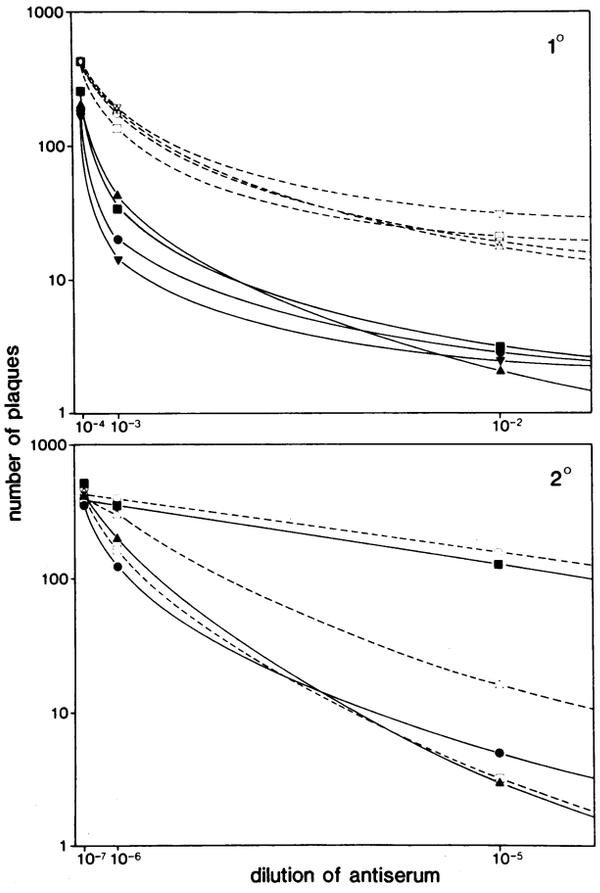

FIG. 4. Titration curves of DNP-haptenated phage inactivation by antisera from control and κ suppressed mice. Note the different scales of dilution of the primary (*Upper*) and secondary (*Lower*) response. —, Antisera from individual control mice injected with normal rabbit IgG from birth; ----, antisera from individual κ suppressed mice.

would expect to obtain antibodies as well against the protein part of the antigen if the mice were able to produce them. Table 1 summarizes the hemagglutination data of anti-DNP antisera from κ suppressed or control mice tested for activity against the carrier used. Experiment I shows that a κ suppressed mouse can make a considerable immune response against KLH when hyperimmunized. A significant secondary response against CGG was also detected. In experiment II, the anti-KLH response of mice immunized with DNP-KLH was investigated in more detail. (Antisera are the same that were analyzed for anti-DNP antibodies in Fig. 4.) A pattern similar to that of the influenza response was found. During the primary response, antibodies to KLH were below the level of detection, whereas in the secondary response high titers of anti-KLH antibodies were found.

Table 1. Titration of anti-DNP antisera from κ suppressed and control mice for reactivity with the carrier used for immunization

Treatment of animal*	Antigen	Titer† Preimmune	1°	2°
	Experiment I			
Untreated	KLH	<20‡	ND	1,600
Suppressed	KLH	<20	ND	800§
Untreated	CGG	<10	ND	320
Suppressed	CGG	<10	ND	80
	Experiment II			
Untreated	KLH	<20‡	320	40,000
	KLH	<20	640	>40,000
	KLH	<20	640	40,000
Injected with normal rabbit IgG	KLH	<20¶	640	>40,000
	KLH	<20	640	>40,000
	KLH	<20	320	40,000
Suppressed	KLH	<20‖	<20	2,500
	KLH	<20	<20	1,280
	KLH	<20	<20	5,000
Standard-isolated anti-KLH antibody**	KLH	1,600	800	1,600

ND, not determined.
*Coupling method: experiment I, tannic acid (19); experiment II, chromium chloride (20).
†Titers are the reciprocal dilutions of the endpoint of hemagglutination. Preimmune, serum prior to immunization; 1°, primary response antiserum; 2°, secondary response antiserum.
‡A pool of normal sera from Balb/c mice was used instead of the actual preimmune sera.
§This mouse received four injections of DNP-KLH.
¶Normal sera from individual mice injected with rabbit IgG from birth; these sera are not preimmune sera of the mice used for immunization. Titers of 1° and 2° antisera in a horizontal line are from the same individual.
‖Titers of preimmune serum and 1° and 2° antisera in a horizontal line are from the same individual.
**Isolated anti-KLH antibodies (a gift of P. Burrows) were used for standardizing titrations done at different times.

DISCUSSION

For the establishment of suppression of κ light chain expression, we have found it essential that anti-κ, in addition to anti-μ, be present in the parental females. Administering only anti-μ in the parental generation resulted in partial suppression (27) or no suppression at all (this paper). Mice, although completely suppressed for IgM expression, display some IgG and IgA in their serum (21). Because μ chain-bearing cells are believed to be the precursors of all B cells, it was postulated that a by-pass might exist in which μ chain-expressing pre-B-cells switch directly to γ chain-expressing cells without synthesizing light chains or even rearranging the light chain genes (21). In fact, a cell line was recently described exhibiting exactly that feature (28). The anti-κ is expected to inhibit or delay this by-pass, and indeed our IgM suppressed mice displayed lower κ chain levels than expected from the literature (21, 22). Therefore, κ light chain immunoglobulin, also, must be lower in fetuses and newborns.

Further, the doses of anti-κ given to the parental females might be high enough to be in excess in the pregnant female for a short while and, therefore, also in the serum of the fetus. Because B cells from neonatal mice are known to be more susceptible to the establishing of tolerance and to anti-immunoglobulin treatment (29–31), at this time, the short pulse of anti-κ might be sufficient to wipe out all κ chain-bearing cells.

Once κ chain suppression is established, it should be stable because no by-pass is possible. This expectation was met because suppression was stable longer than 1 year, provided the anti-κ injections were continued. In addition, suppression could be easily maintained over several generations.

This need for continuous administration of anti-κ suggests that suppression is not due to an active mechanism such as suppressor T-cells but merely to the depletion of mature B cells expressing κ chain on the surface. The precursors for these cells are not affected because, soon after the injections are stopped, immunoglobulin starts to appear in serum and

because bone marrow cells from normal and suppressed animals were indistinguishable with regard to secretion after LPS stimulation *in vitro*. In spleen also, a few κ chain precursors seem to be present. In all these respects, κ suppression closely resembles μ suppression (21, 32).

The most interesting feature of κ suppressed mice is that the lack of κ chain expression is compensated by an enhanced amount of λ chain-bearing immunoglobulins and cells. This phenomenon is well established in rabbits that lack κ chains because of allotype suppression (33) or a genetic defect (10). Similar to the rabbit, the level of the molecules not suppressed remains high even after suppression is broken (33). Because no obvious regulatory cells seem to be involved in this form of suppression, certain clones of λ chain-expressing lymphocytes directed against environmental antigens must achieve an advantage over κ chain-bearing lymphocytes, which would normally be stimulated preferentially. The dominance of λ chain clones is maintained even after κ chain is allowed to be reexpressed.

Why is λ chain under normal conditions expressed only in low amounts? The answer is that, from two germ-line genes, only a limited number of somatic variants can be generated in a given time. Because the κ chain pool starts from more than 100 *V* genes (7), fewer mutational steps will be required for a particular lymphocyte to gain good antigen-specificity. The chance is, therefore, higher that a κ chain-expressing lymphocyte will be stimulated during an immune response. Even under our conditions where competition with κ chain-bearing cells cannot take place, only very limited heterogeneity is found among the λ_1 light chains (Fig. 3B) and even less was detected in λ_2 chains. This seems to be reflected in the fact that eyebleeding resulted in severe infections, the primary response being lower than normal against DNP and not measurable against influenza strain A and KLH. On the other hand, the anti-κ solution was not sterile and sometimes was stored for 1 or 2 months at +4°C without any effect on the mice. Although the mice were kept on antibiotic water routinely, this precaution seemed not to be essential. The secondary responses to DNP and influenza were quite normal, and the anti-KLH response was reasonably high compared to control mice. Although this requires further investigation, it suggests that a mouse has a large potential repertoire of antibodies carrying λ light chains.

Mice suppressed for κ light chain expression offer a unique model system for studying the potential of a limited number of well-characterized germ-line *V* genes with regard to their repertoire, their mutational sites, and their interaction with the heavy chain pool. In addition, this model system offers the opportunity to investigate the consequences of the lack of most of the normal idiotypic repertoire on the different immunological compartments and on the postulated network of idiotypes (34).

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