A MAJOR GROUP OF MOUSE KAPPA CHAINS CONTROLLED BY THE CHROMOSOME 6 LOCUS, *IgK-Ef2**

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Polymorphism of mouse kappa chains has been shown to be associated with chromosome 6 loci closely linked to the *Ly-2,3* locus (1–5). Because the gene for the constant region of the kappa light chain, and at least certain genes for the variable region of the kappa light chain $(V_\kappa)^1$ are now known to be located on chromosome 6 (6, 7), the simplest interpretation of the kappa chain genetic differences is that they are a result of differences in the V_κ genetic complex itself. Precise characterization of the genetic differences at both the protein and nucleic acid level could help define the number of light chains controlled by each V_κ gene. For example, it is now apparent that many light chains may be produced from each V_κ gene simply by combinatorial association with different J_κ segments (8–11). In addition, each V_κ gene may give rise to an as yet unknown number of somatic mutants, by normal mutation rates followed by clonal selection of useful variants in the course of immune responses (12). Although it is likely that both of these mechanisms are important in generating antibody diversity, the extent to which they actually do occur could be gauged if one were able to characterize the set of gene products generated by a given V_κ gene. Genetic polymorphism involving differences of a few V_κ genes could provide an invaluable tool in this type of analysis.

We have concentrated our attention on the structural differences controlled by the *IgK-Ef2* locus (5). This locus controls differences in normal light chain isoelectric focusing (IF) patterns, and it has been shown to be closely linked to two chromosome 6 markers, *Ly-3* and *Hd* (5).

An interesting aspect of the Ef2 marker was that it appeared to involve discrete groups of light chains, as evidenced by the disappearance of certain bands from the normal light chain IF profiles of $Ef2^b$ strains, such as NZB mice, as compared with $Ef2^a$ strains, such as BALB/c. The nature of the IF differences suggested that specific V_κ genes might either be absent in $Ef2^b$ strains or, alternatively, not expressed in serum immunoglobulin of these strains. To approach the question of the nature of the focusing bands seen in normal light chains and, in particular, to obtain information as to the identity of the polymorphic light chain bands, we screened a large number of BALB/c and NZB myelomas in search of light chains that cofocus with the major normal light chain IF bands affected by the *IgK-Ef2* locus. This survey led to the

[1] *Abbreviations used in this paper*: DNP, dinitrophenyl; HPLC, high-performance liquid chromatography; IF, isoelectric focusing; PBS, phosphate-buffered saline; PTH, phenylthiohydantoin; TFA, trifluoroacetic acid; V_κ, variable region of the kappa light chain.

identification of 8 BALB/c myelomas (out of a total of 277 examined) that have light chains indistinguishable from the marker light chain bands. In contrast, no light chains that cofocused precisely with the marker bands were identified among a total of 133 NZB myelomas (5).

We have now obtained partial amino acid sequences of 3 of the BALB/c light chains in question, and all 3 have been found to share an identical amino acid sequence over the first 30 residues. This result provides strong support for the notion that at least some of the discrete focusing bands (IF bands) observed in normal serum immunoglobulin light chains represent light chains that belong to the same V-region group (if not subgroup). It also suggests that the genetic polymorphism may be a result of the absence of a single V_κ gene or of a cluster of closely related V_κ genes in $Ef2^b$ strains of mice. The surprisingly high frequency of light chains of the Ef2-related group among BALB/c myelomas suggests that it may represent one of a small number of V_κ groups expressed at an elevated level.

Materials and Methods

Mouse Myeloma Ascites Fluids.   Samples of BALB/c myeloma ascites fluids selected at random from the BALB/c myeloma collection were generously supplied by Dr. Michael Potter of the National Cancer Institute (Bethesda, Md.). Samples of myeloma ascites fluids of NZB mice were selected only for being good producers and were otherwise a random sample. These samples were generously donated by Dr. Martin Weigert of the Institute for Cancer Research, Philadelphia, Pa.

Production of large amounts of immunoglobulin from the BALB/c tumors TEPC-817, FLOPC-1, and TEPC (CAL-20)-105 was undertaken at the University of Sherbrooke (Sherbrooke, Canada) using pristane-primed BALB/cByJ mice or CAL-20 mice obtained from Dr. Judy Wax at Litton Bionetics, Kensington, Md.

Isolation of Immunoglobulins and Light Chains.   Ascites fluids were delipidated then diluted with an equal volume of phosphate-buffered saline (PBS). A vol of 3.5 M ammonium sulfate, pH 7.3, equal to the diluted volume was then added, and precipitation was allowed to proceed for 1 h at 0°C. The precipitate was redissolved in twice the original fluid volume of PBS, and the precipitation step was repeated. After three precipitations, the immunoglobulin was redissolved, dialyzed against dilute ammonium bicarbonate, and lyophilized. Immunoglobulin was subjected to partial sulfityolysis and heavy and light chains separated by gel filtration on Sephadex G100 (Pharmacia Fine Chemicals, Div. of Pharmacia, Inc., Piscataway, N. J.) in the presence of 0.1% sodium dodecyl sulfate as described (13). Light chains were then desalted on Sephadex G25 in ammonium carbonate and lyophilized. All light chain preparations were rechromatographed a second time on Sephadex G100. An alternative procedure, used for small preparations of light chain, was partial reduction and alkylation with iodoacetamide followed by gel filtration on Ultragel ACA 34 in the presence of 3 M guanidine HCl and 0.2 M ammonium bicarbonate (14).

Sequenator Procedures.   A 1-M quadrol program was used (15) on a modified Illitron model 9001 sequenator (Illitron, Division of Illinois Tool Works Inc., Chicago, Ill.). Program modifications, such as solvent precipitation steps (16), were also incorporated. Conversion of thiazolinone amino acid derivatives was done manually, using 25% trifluoroacetic acid (TFA) at 60°C for 20 min. After drying of the TFA, the entire residue was dissolved in 0.2 ml of methanol: acetonitrile (50:50) for injection into the high-performance liquid chromatograph (HPLC). Phenylthiohydantoin (PTH) amino acids were identified by HPLC using a Waters solvent programmer and delivery system, dual wavelength detector (254 and 313 nm) (Waters Associates, Inc., Milford, Mass.) and a Dupont 4.6-mm × 25-cm Zorbax CN column (E. I. DuPont de Nemours & Co., Inc., Wilmington, Del.). Conditions of elution were similar to those described by Johnson et al. (17). The gradient was from 0 to 55% solvent B (15% acetonitrile in methanol) using curve 2 over 20 min. Solvent A consisted of 0.0167 M sodium acetate buffer (pH 4.7). All solvents were millipore filtered. Solvent A was also sterilized by autoclaving. In

addition to HPLC, in certain instances, residues were hydrolyzed and analyzed as free amino acids using a Technicon TSM-1 amino acid analyzer (Technicon Instruments Corp., Tarrytown, N. Y.) (18).

IF. Procedures for purification of normal immunoglobulin, light chain isolation, and IF of normal light chains have been described (3, 5). For the analysis of myeloma ascites fluids, 1 µl of fluid was subjected to complete reduction and alkylation with [^{14}C]iodoacetamide (50 mCi/ mmol; Amersham Corp., Arlington Heights, Ill.), as described. The reduced and alkylated material was directly loaded on the urea formate gel for chain separation. Because no purification of the myeloma immunoglobulin was carried out, the myeloma light chain patterns were often contaminated by extraneous minor bands. In all cases the myeloma light chain bands were unequivocally identifiable as major unique bands.

Results

Differences Expressed in Normal BALB/c and NZB Light Chains. Normal light chain IF spectra of NZB and BALB/c mice are illustrated in Fig. 1. Differences in the region of bands 54 and 63 are among the most prominent, although other distinct differences are also evident (e.g., bands 5 and 20). One of the eight BALB/c myeloma light chains corresponding to the bands 54/63 of the normal pattern is also illustrated (Fig. 1, position d). Fig. 2 shows seven of the myeloma light chains cofocusing with the BALB/c bands 63 and 54. One of these myelomas (TEPC-602) displays a second, probably unrelated, light-chain band.

N-Terminal Sequences of Ef2-related Light Chains. Three of the BALB/c myeloma light chains corresponding to the Ef2 marker bands were each sequenced at least twice over the N-terminal 40 residues. Unequivocal sequences were obtained for each of the proteins for the first 30 positions using a combination of HPLC and back hydrolysis procedures. Sequence data obtained for the light chain TEPC-817 is illustrated in Fig. 3. The sequences of the three proteins were indistinguishable from each other and were identical to that of the light chain of MOPC-460, a well-characterized dinitrophenyl (DNP)-binding myeloma protein (19). According to the classification of Potter (20), these sequences belong to the $V_\kappa 1$ group. A summary of sequences of light chains closely related to $V_\kappa 1$ is given in Table I.

Closely Related Light Chains Are Present in NZB Mice. Examination of the available N-terminal sequence data for BALB/c and NZB kappa chains (21) indicated that two light chains with a sequence identical to that of the Ef2 group in the first 23 positions have also been found in NZB myelomas (Table I). This was somewhat surprising because it was expected that the group (in this case, $V_\kappa 1$) representing the polymorphic bands would be reduced considerably, if not absent, in NZB, an *IgK-Ef2^b* strain. The two NZB light chains in question—namely, PC2205 and PC2567— were clearly distinct from the Ef2 group on IF however, which indicated that they must differ structurally from the BALB/c proteins beyond position 23 in the variable region (Fig. 1 g and h). It is relevant to note in this context that members of the $V_\kappa 21$ group all share an identical sequence in the region of 1–23, but at least six subgroups ($V_\kappa 21A$, -B, -C, -D, -E, and -F) are evident when the entire V-region sequence is considered (10). IF analysis also divides the $V_\kappa 21$ group into at least six distinguishable subgroups, more in line with the results of total sequence analysis.

Relationship to MOPC-460 Light Chain. All three of the Ef2 marker light chains showed an amino acid sequence identical to that of the light chain of the BALB/c myeloma protein MOPC-460 over the first 30 positions, thus indicating that these light chains must fall into the same subgroup or closely related subgroups. IF of the
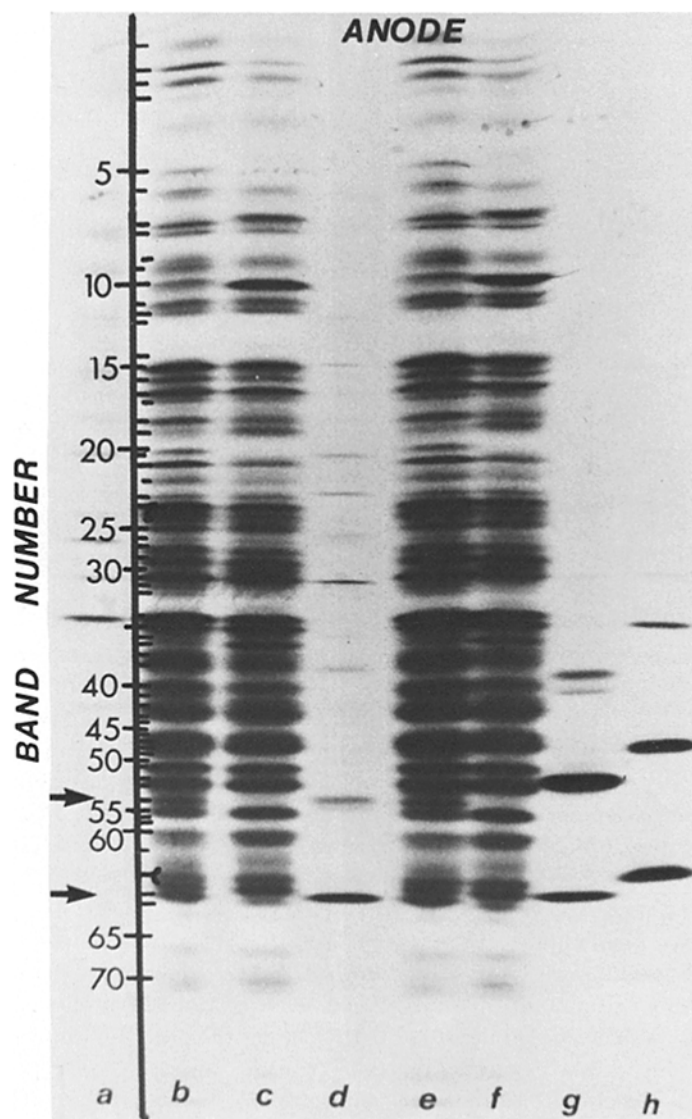
Fig. 1. IF profiles of normal serum immunoglobulin light chains and myeloma light chains. Normal serum light chains of NZB mice are shown in channels c and f. Normal light chains of BALB/c mice are shown in channels b and e. Light chains of the Ef2-related BALB/c myeloma FLOPC-1, is in channel d. Structurally related myeloma light chains of MOPC-460 and NZB myelomas PC2205 and PC2567 are in channels a, g, and h. Arrows indicate the major Ef2-related differences in the NZB and BALB/c light-chain profiles. The figure represents a 1-wk autoradiogram of the dried gel. Arbitrary band numbers have been assigned simply to aid reference to the text and to Table I.

MOPC-460 light chain showed that it was clearly distinct however, because it focused ~3 charge U anodal to the Ef2 light chains (Fig. 1 a). Whether or not MOPC-460 light chain may be considered to be a somatic mutant of the Ef2-related group is an interesting question and one that could better be answered when the complete amino acid sequences of these light chains are available.
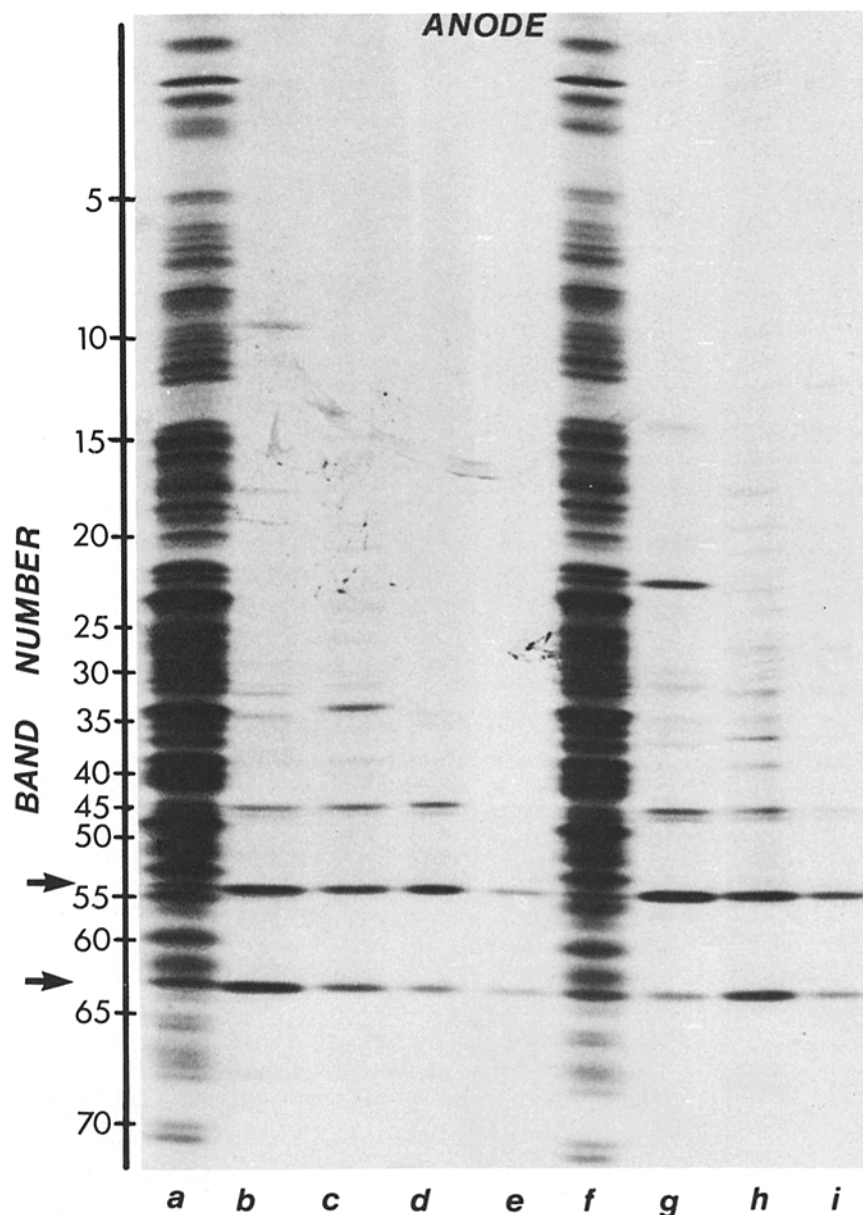
Fig. 2.   IF profiles of Ef2-related BALB/c myeloma light chains. Normal BALB/c light chain is shown in lanes a and f. Ef2-related myelomas TEPC-817, TEPC-(CAL-20)-105, TEPC-821, TEPC (CAL-20)-119, TEPC-602, FLOPC-1, and FLOPC-21 (G2) are shown in lanes b, c, d, e, g, h, and i.

Discussion

In these studies, we believe we have identified one of the important V_κ groups involved in the Ef2 polymorphism previously described (5). Sequencing of the first three of the eight BALB/c myelomas found to possess light chains corresponding to the Ef2 marker bands (63 and 54) revealed an identical sequence over the first 30
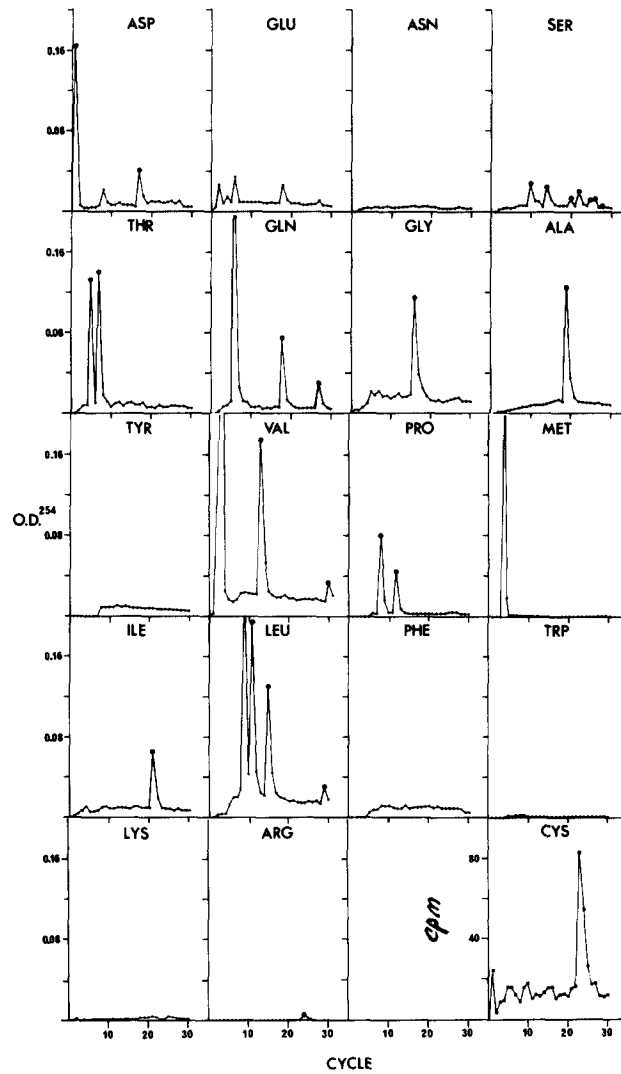
Fig. 3.   Recoveries of PTH amino acids in the automated degradation of the light-chain TEPC-817.

residues. The probability of selecting three proteins having sequence identity in the first 30 positions by chance is extremely low, so that the result, in itself, adds credibility to interpretations of light chain IF data. It seems likely from this result that the majority of the remaining five myelomas identified will also possess the same N-terminal sequence. Complete sequencing of these light chains is under way to determine whether they constitute a single subgroup or whether they represent several closely related subgroups. The frequency of occurrence of the Ef2-related light chains (8 out of a total of 277 myelomas examined) suggests that the group must be one of a relatively small number of groups. According to the Poisson formula, if all samples fell into one of 100 equal groups, the expected proportion of groups represented 8 or more times would be 0.0066. If there were 50 groups, the expected proportion

TABLE I

*N-Terminal Sequences of Light Chains Related to the Ef2 Marker Bands**

IF bands	Light chain	1	5	10	15	20	25	30	Strain	Reference
34. 26	MOPC-460	D V V M T	Q T P L S	L P V S L	G D Q A S	I S C R S	S Q S L V		BALB/c	Barstad et al. (19)
	J509	___ L __					__ I _		BALB/c	Barstad et al. (19)
63. 54	TEPC-817								BALB/c	This work
63. 54	FLOPC-1								BALB/c	This work
63. 54	TEPC (CAL-20)-105								BALB/c	This work
62.5. 52	2205								NZB	Loh et al. (21)
62. 49	2567								NZB	Loh et al. (21)

* BALB/c myelomas corresponding to Ef2 marker bands 54 and 63 include TEPC-817, FLOPC-1, TEPC (CAL-20)-105, TEPC-602, TEPC-821, TEPC (CAL-20)-119, FLOPC-21 (G2), and RAW-124.

represented 8 or more times would be 0.178, a more reasonable probability. This calculation would therefore suggest that the number of groups of light chains similar to the Ef2 group would be closer to 50 than to 100. Whether or not the sequences represented in the myelomas are a valid sampling of the total repertoire is of crucial importance in considering extrapolation of these calculations to the total number of light-chain groups. This point has been raised by Loh et al. (21) in their analysis of the sequences of randomly selected BALB/c and NZB kappa chains. Our results indicate that the proportion of Ef2-related light chains in normal serum immunoglobulin and artificially reconstituted mixtures of myeloma proteins is similar, as judged by the relative intensities of the bands on autoradiograms of IF gels.

In considering the implications of the present results for our interpretations of the genetically controlled light-chain polymorphism, the central assumption we must make is that the V_κ group defined by the cofocusing myelomas is, in fact, the same V_κ group that we observe in normal serum light chains (identified as bands 63 and 54). We feel this assumption is valid for the following reasons: First, the selection of three light chains identical over the first 30 positions simply by the criterion of IF dramatically demonstrates the validity of the method as a means of identifying sequence-related light chains. There is no reason to doubt that the same degree of selectivity is also obtained when the method is applied to the normal light-chain pool and, therefore, that light chains in the normal pool showing close sequence relationships will focus together as a group or band. Secondly, the finding that three closely related light chains cofocus precisely with the normal light-chain bands 54 and 63 also indicates that a bona fide V_κ sequence group corresponding to these bands does, in fact, exist among BALB/c myeloma immunoglobulins. It follows that if such light chains exist in normal serum immunoglobulin they will also cofocus with the marker bands 54 and 63.

The fact that discrete groups of light chains are controlled by the *IgK-Ef2* locus is more readily explained by proposing that the polymorphism involves structural V_κ genes rather than regulatory loci. Regulatory loci such as that described for the λ_1 light-chain subgroup do, nevertheless, exist (22), and one could suppose that similar loci might control kappa subgroups as well. This would necessarily be more complicated for kappa chains because there may be on the order of 300 V_κ subgroups (23), which would imply the existence of multiple regulatory loci. Other Ly-2,3-linked V_κ loci such as *IgK-Ef1* (24), *IgK-Pc* (25), and *IgK-Trp* (26), each of which appears to control structurally related subsets of kappa chains, could also be supposed to

represent V_κ regulatory loci. One weakness of the argument for regulatory loci is that it implies some functional significance for each control group of light chains. Because light chains of each V_κ subgroup are believed to associate with a wide variety (if not all possible) heavy chain variable region partners to give different functional combinations, the existence of a specific function for individual V_κ genes is unlikely. If it is assumed that the polymorphism is a result of differences in content of V_κ genes or in sequences adjacent to V_κ genes directly affecting their expression; the present results would suggest that mice carrying the $IgK-Ef2^b$ allele have defective or missing genes corresponding to the V region of TEPC-817, FLOPC-1, TEPC (CAL-20)-105, etc. ($V_\kappa1$). Because extensive gene duplication and deletion must have occurred during the evolution of immunoglobulin V regions (27–29), it would not be too surprising to find polymorphism of gene numbers within the inbred strains of mice (30). The fact that multiple differences in normal light-chain IF patterns are controlled by $IgK-Ef2$ would suggest that several V_κ subgroups may be involved. Identification of other subgroups of light chains affected by $IgK-Ef2$ by further analysis of the BALB/c and NZB myeloma collections could be useful from the point of view of genetic mapping of the V_κ locus. One would expect that subgroups that were simultaneously deleted by unequal crossing over would be close together on the chromosome. Similar analysis could also be made of the more extensive differences associated with the $IgK-Ef1$ polymorphism (3).

The finding that one of the major groups of light chain controlled by $IgK-Ef2$ is closely related to the MOPC-460 light chain has implications in terms of the anti-DNP antibody response. Because it is known that MOPC-460 has a high affinity for DNP (31) it will be interesting to examine light chains produced in the DNP response in strains of mice differing with respect to $IgK-Ef2$. We are currently engaged in these studies. It is of great interest to note that others have found that $Ef2^b$ strains such as C58 are unable to produce anti-DNP antibodies carrying the MOPC-460 idiotype (32). It seems likely from this result that $Ef2$-controlled light chains may well be involved in the anti-DNP immune response and, in particular, that they may be the subgroup required for expression of the MOPC-460 idiotype.

It is clear that resolution of the question of whether the light-chain polymorphism involves regulatory or structural genes will require direct examination of the structural genes. The identification of a major structurally related set of light chains controlled by the $IgK-Ef2$ locus will greatly aid in this analysis. It should be noted though that sequences closely related to the polymorphic group are also found in $IgK-Ef2^b$ strains (e.g., NZB), and this could complicate attempts to directly demonstrate the absence of given V_κ structural genes at the DNA level.

Summary

We previously demonstrated that loci closely linked to the $Ly-3$ locus control the expression of distinct sets of light chains in normal mouse serum immunoglobulin. One of these loci, $IgK-Ef2$, was shown to control two major bands in normal light chain isoelectric focusing (IF) profiles. Strains possessing the marker bands were designated $IgK-Ef2^a$. Screening of myeloma proteins from the strains BALB/c ($IgK-Ef2^a$) and NZB ($IgK-Ef2^b$) led to the identification of eight proteins in the BALB/c collection having light chains that cofocus precisely with the polymorphic IF bands observed in normal serum light chains. Partial sequence analysis of 3 of the light

chains has shown that they are all identical in the first 30 positions, which indicates that they constitute a single variable region of the kappa light chain (V_κ) group ($V_\kappa 1$). The frequency of occurrence of the group within the BALB/c myeloma collection (8 out of 277) suggests that the number of such groups may be closer to 50 than to 100. The finding supports an interpretation of the genetic polymorphism as being in part a result of the absence of genes related to $V_\kappa 1$ in *IgK-Ef2*^{b} strains of mice.

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