Genetic Origins of B and T Cell Antibodies

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I. INTRODUCTION

The immune system is the vertebrate's principal means of body defense against pathogens, such as bacteria and viruses, as well as the organism's own cells that have undergone cancerous transformation. The essential principle of the system is pattern recognition. The protein molecules responsible for the recognition are called antibodies while the proteins, carbohydrates, and other entities recognized by antibodies are referred to as antigens.

Two types of immunity occur: humoral and cellular. The antibodies mediating the humoral immunity are immunoglobulins (Ig's) synthesized by bone marrow-derived (B) lymphocytes while the antibodies responsible for the cellular immunity are synthesized by the thymus-derived (T) lymphocytes. We have extensive functional and structural information on Ig's but we remain virtually ignorant about the structure of the T cell antibody, i.e., antigen receptors on T cells.

Since antigens are structurally diverse, the repertoire of antibodies of a single organism must be very large. How the genetic information needed for this large set of antibody molecules is stored in a genome and inherited through generations has been one of the central issues in immunology and genetics. The application of recombinant DNA technology has now resolved this issue, at least in its basic outlines, for the B cell antibodies. Results have shown that the Ig genes are altered somatically during the development of lymphocytes both by recombination and mutation. This chapter

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presents new data on mutations of mouse κ light chain genes, summarizes the current state of knowledge on somatic mutations in Ig genes in general, briefly reviews the essence of the somatic recombination in these genes, and, finally, describes the results of the initial experiment that may lead to the elucidation of the T cell receptor mystery.

II. SOMATIC MUTATIONS INCREASE DIVERSITY OF THE SINGLE GERMLINE MOUSE $V_{\lambda 1}$ GENE SEGMENT

An antibody molecule is composed of two identical light chains and two identical heavy chains. The amino acids primarily responsible for recognition of an antigen reside in the amino terminal regions referred to as variable regions (V regions). Light chains appear as two types, κ and λ. In the mouse the λ chains are further divided into three subtypes, λ1, λ2, and λ3. The ratio of light chains contained in the serum Ig molecules varies, depending on mammalian species. In mice this ratio is heavily skewed toward κ chains and only about 5% of the serum Ig molecules contain λ chains.

As expected from this result, the V region heterogeneity of mouse λ chains is relatively limited. Weigert, Cohn, and their co-workers determined the amino acid sequences of the V regions of 18 randomly chosen λ1 chains synthesized by 18 independently induced BALB/c mouse myelomas (Weigert *et al.*, 1970). They found that 12 out of the 18 $V_{\lambda 1}$ regions have an identical sequence which we refer to as the prototype sequence. The other six $V_{\lambda 1}$ regions are very similar to the prototype sequence but differ in one to three residues. In all but one case the codons of the variant residues can be generated from the codons of the corresponding residues of the prototype sequence by a single base change. They, therefore, guessed that the mouse has a single germline $V_{\lambda 1}$ gene which codes for the prototype sequence and the variant sequences arise by somatic mutations.

Several years ago we tested this hypothesis by using restriction enzymes and recombinant DNA technology and proved that the mouse indeed has a single germline $V_{\lambda 1}$ gene (Brack *et al.*, 1978; Bernard *et al.*, 1978). We, therefore, concluded that somatic mutation must amplify the genetic information in the germline $V_{\lambda 1}$ gene.

III. SUBSET OF MOUSE κ CHAIN V REGIONS (V_κ-21 GROUP) ENCODED BY ABOUT TEN GERMLINE V_κ GENE SEGMENTS

Recently, we extended this work to κ chains. In mice, the V_κ regions are more heterogeneous than V_λ regions. Potter classified mouse κ chains to groups, based on a rather arbitrary criteria: namely, if two V_κ regions are

different in two or more positions in the amino terminal 23 residues they belong to two different groups (Potter, 1977). The V_κ regions were further classified to subgroups based on sharing or nonsharing of subgroup-specific residues. McKean *et al.* (1978) and Weigert *et al.* (1978) focused their efforts to one of the V_κ groups, V_κ-21, and determined the entire or nearly entire V region sequences of over 30 myeloma chains of BALB/c and NZB origins. Their studies lead to identification of seven V_κ-21 subgroups: V_κ-21A to V_κ 21G. The amino acid sequence variability within a V_κ-21 subgroup is similar to that of the $V_{\lambda 1}$ regions. Namely, two or more κ chains of independent myeloma origins give an identical V region sequence (prototype sequence) and the rest of the κ have unique V region sequences, each of which differs from the prototype sequences by one to several substitutions (Fig. 1).

As expected from the fact that the mouse V_κ regions are much more heterogeneous than $V_{\lambda 1}$ regions, the mouse carries multiple V_κ genes in the germline genome (Lenhard-Schuller *et al.*, 1978; Seidman *et al.*, 1978). This can be demonstrated by the Southern gel blot analysis of total cellular DNA using a V_κ gene probe. As shown in Fig. 2, V_κ probe isolated from myelama MOPC 321 (belongs to Vκ-21) detects eight DNA bands (the fastest moving is a double band) when analyzed after digestion with one of less frequently cutting restriction enzymes. The V_κ genes residing in these DNA fragments should represent most, if not all, of the V_κ-21 germ line genes. Experimental evidence supporting this contention was obtained earlier by Valbuena *et al.* (1978). The V_κ regions of myelomas PC 2880 and MOPC 321 show the least degree of sequence homology among all known pairs of κ chains belonging to the V_κ-21 group (Weigert *et al.*, 1978). In spite of this, 92% of the sequence in the full length κ cDNA from MOPC 321 could be protected against S1 nuclease digestion when it was preannealed with excess κ mRNA from PC 2880.

We cloned the *Eco*RI fragments detected by the MOPC 321 V_κ probe, mapped the positions of the V_κ-21 genes on the cloned DNA fragments, and determined their nucleotide sequences. As shown in Fig. 2, all DNA fragments except for the 18 kb fragment carry one copy of the V_κ-21 genes, the 18 kb bearing two copies. Independent isolates were characterized for most of the bands. Except for the 9 kb band, which actually contained two fragments of 9.5 and 8.5 kb, all bands represented a single fragment type. Altogether, eleven V_κ-21 germline genes were identified, of which one, that on the 4 kb *Eco*RI fragment, apparently has an insertion in the middle of the gene, and is, therefore, probably an inactive gene.

IV. V_κ-21 GENE SEGMENTS ARE CLUSTERED

We attempted to determine the relative positions of the eleven V_κ-21 genes by cloning overlapping DNA fragments from a library of embryo

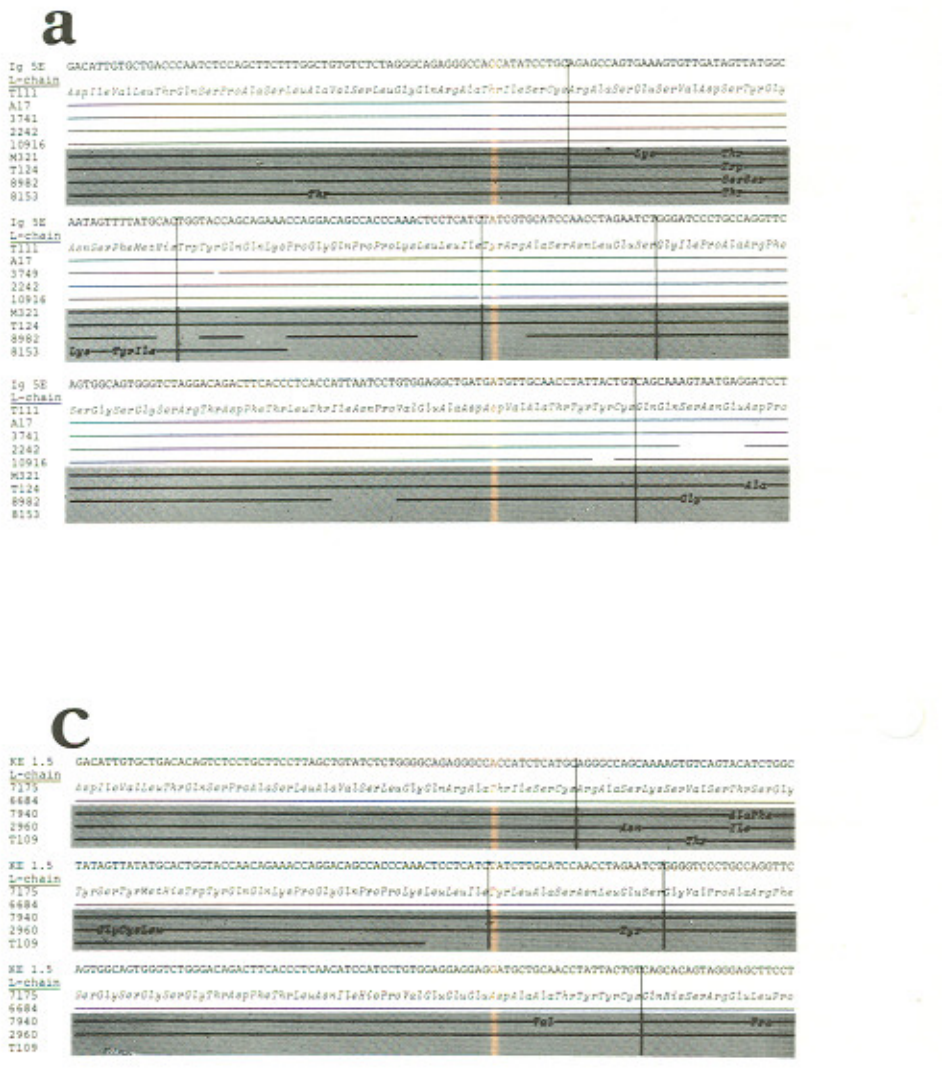
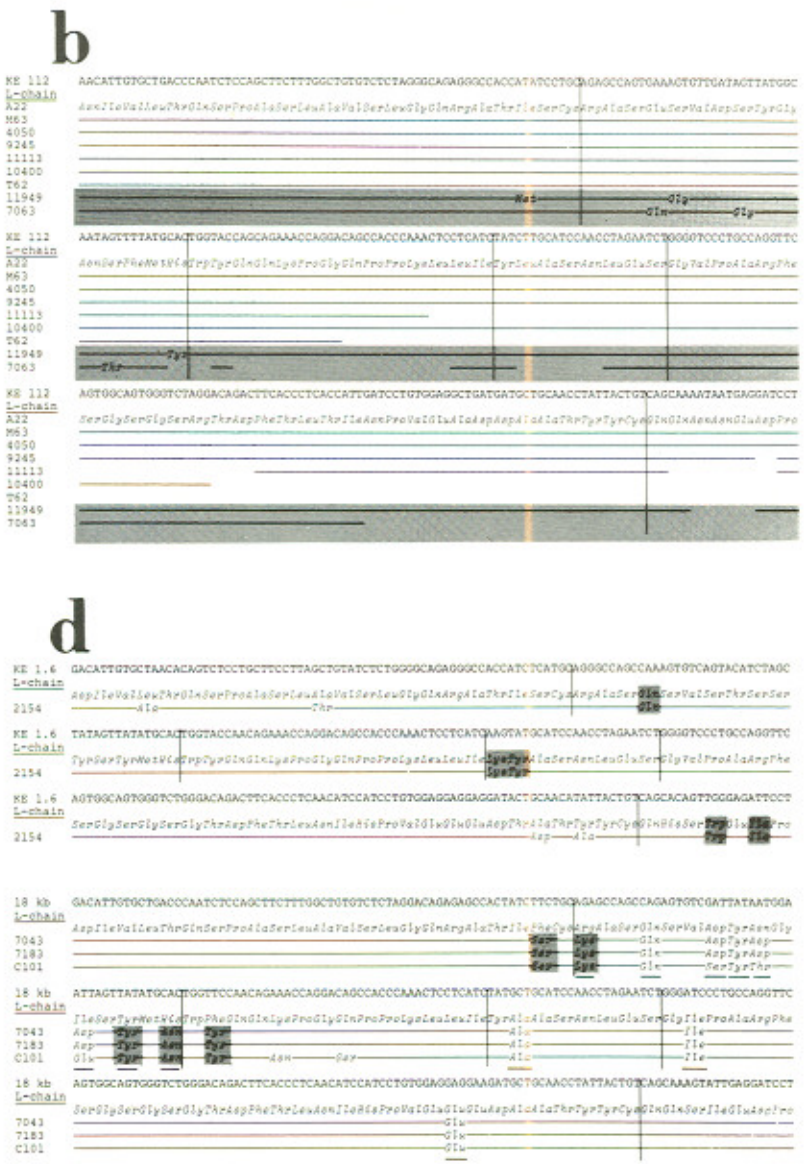


Fig. 1. Comparison of the germline V_κ nucleotide sequences with the amino acid sequences of V_κ-21 group. (a) The nucleotide sequence of the 9.5 kb V clone, Ig 5E, is compared with the amino acid sequences of eight V_κ regions belonging to V_κ-21C subgroup. The nucleotide sequence corresponds exactly to the prototype amino acid sequence carried by myelomas, T111, A17, 3741, 2242, and 10916. The amino acid sequence data are from Weigert *et al.*(1978), McKean *et al.* (1978), and personal communications from M. Weigert. Variant amino acid sequences are indicated by shading. Vertical lines indicate the boundaries of FWR's and CDR's. (b) The nucleotide sequence of the 16 kb *V* clone, KE112, is compared with the amino acid sequences of 9 V_κ regions belonging to V_κ-21B sub-



group. (c) The nucleotide sequence of the 1.5 kb *V* clone, KE 1.5, is compared with the amino acid sequences of 5 V_κ regions belonging to V_κ-21E subgroup. (d) In the upper panel the nucleotide sequence of 1.6 kb *V* clone, KE1.6, is compared with the amino acid sequence of the V_κ region of myeloma 2154. In the lower panel the nucleotide sequence of the 5′ 18 kb *V* gene is compared with three V_κ regions.

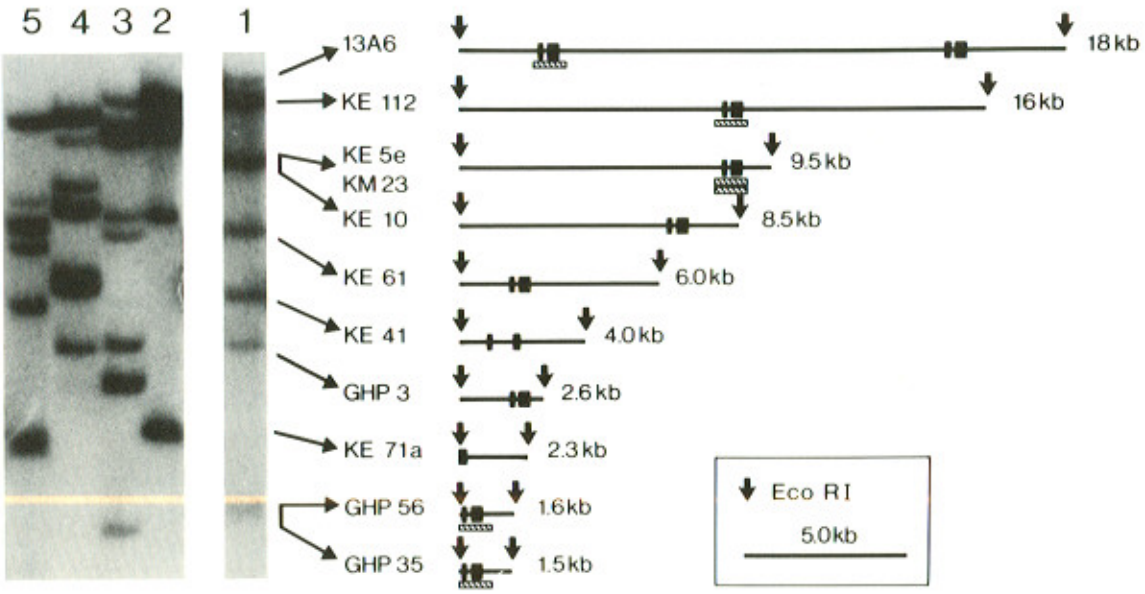


Fig. 2. Southern blot analysis of BALB/c embryo DNA and DNA clones isolated from the detected bands. (Left panel) embryo DNA was digested with (1) *Eco*RI, (2) *Hpa*I, (3) *Bgl*II, (4) *Hin*dIII, or (5) *Bam*HI, and analyzed with a probe consisting of the 850 bp *Hha*I insert of plasmid 5DIO (Lenhard-Schuller *et al.*, 1978). (Right panel) DNA clones isolated from the *Eco*RI bands are shown. Boxes represent V_κ DNA sequences.

DNA predigested to a limited extent with *Eco*RI. As shown in Fig. 3, this experiment lead to the identification of two V_κ-21 gene clusters. One cluster is about 90 kb long and contains six V_κ-21 genes in the same orientation. The other cluster is about 30 kb long and contains three V_κ-21 genes also in the same orientation. In addition, a series of Southern gel blot experiments suggest that the 30 kb cluster lies 3′ to the 90 kb cluster (results not shown).

V. SOMATIC MUTATIONS AMPLIFY DIVERSITY OF *V* GENE SEGMENTS

The nucleotide sequence of the three V_κ-21 genes, 9.5 kb V_κ, 16 kb V_κ, and 1.5 kb V_κ, are shown in comparison with the V_κ amino acid sequences of subgroup C, subgroup B, and subgroup E, respectively, in Fig. 1. In all three cases the germline nucleotide sequences correspond exactly to the amino acid sequences of the prototype κ chains.

Two more V_κ-21 genes were also sequenced but neither of them (1.6 kb V_κ and 5′ 18 kb V_κ) corresponded to any known V_κ-21 prototype or variant sequences. However, the 1.6 kb V_κ gene sequence shows a high degree of sequence correspondence to the 2154 κ chain (Fig. 1). This myeloma does not belong to any of the seven known V_κ-21 subgroups because no other closely related sequence is known. It is likely that the sequence of this myeloma κ chain represents a variant of the putative prototype sequence encoded by the germline 1.6 kb V_κ gene.

The amino acid sequence encoded by the 5′ 18 kb V_κ gene also does not correspond to any of the known V_κ-21 regions, but shows similarities and differences to the sequence of subgroup D (Fig. 1). Since the known V_κ-21 subgroups are unlikely to be an exhaustive list, we believe that the 5′ 18 kb V_κ gene represents a V_κ-21 subgroup yet to be identified.

Overall results confirm the role of somatic mutations in the amplification of the germline-encoded genetic information for Ig genes. They suggest, at least as a first degree of approximation, that there is one germline V_κ gene for every subgroup. It should be noted that the known mouse $V_{\lambda 1}$ regions constitute one subgroup according to the Potter criteria. As mentioned earlier, these $V_{\lambda 1}$ regions arise from a single germline *V* gene.

Recently, Selsing and Storb (1981) and Gerhsenfeld *et al.* (1981) discovered that a κ cDNA probe prepared from myeloma MOPC 167 atypically detects a single major band in the *Eco*RI digest of kidney DNA. They cloned this germline $V\kappa$ gene and compared its nucleotide sequence with that of κ cDNA clones isolated from MOPC 167. Separate experiments indicate that the κ gene expressed in MOPC 167 arises from the germline V_κ gene (kidney is a surrogate for germline cells). They found that the MOPC 167 cDNA sequence differs from the germline *V* gene sequence by four base pairs.

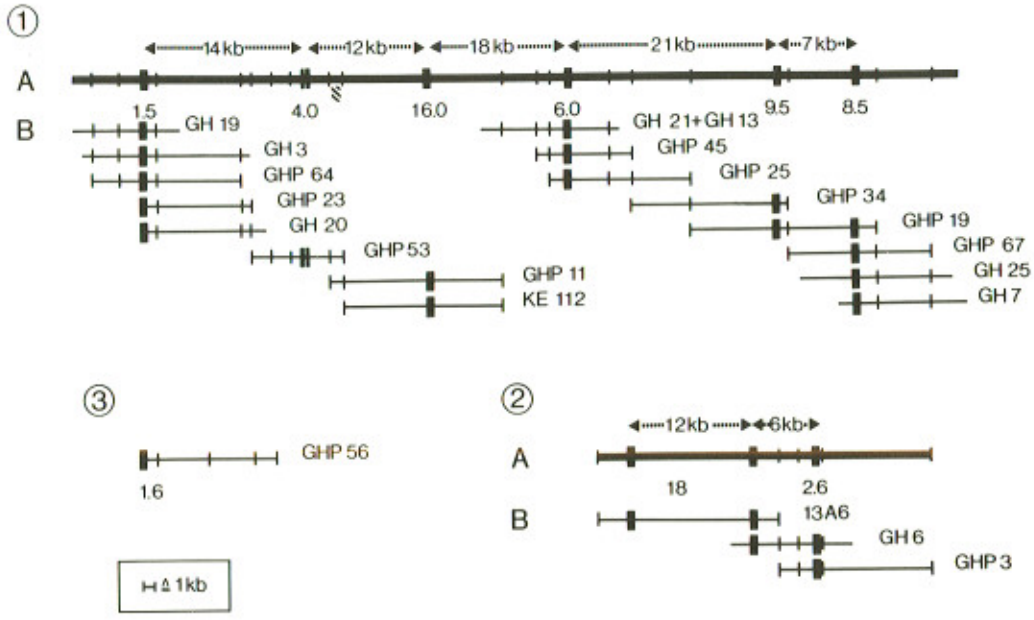


Fig. 3. Linkage map of V_κ-21 germline V_κ DNA segments. The thick horizontal bars represent the linkage maps. Thin horizontal bars are individual clones isolated from a *Eco*RI-partial or *Eco*RI*-partial library of BALB/c embryo DNA. Thin vertical lines are *Eco*RI sites.

VI. SOMATIC MUTATIONS OF HEAVY CHAIN *V* GENE SEGMENTS

For the heavy chain genes the first critical comparison of the *V*-coding DNA sequences of germline and somatic origins was carried out by Sakano *et al.* for the γ2b chain of MOPC 141 (Sakano *et al.*,1980). Two germline sequences, one containing the *L* and *V* DNA segments and the other containing the *J* and *C* DNA segments, and the rearranged somatic sequence were compared for a length of about 650 base pairs composing the *V*, *J*, 5′-flanking, and 3′-flanking regions. They found 12 base substitutions, of which one each was in the 5′-flanking, *L* and *J* regions.

Recently, two more heavy chain gene systems have been studied. Gearhart *et al.* compared the V_H amino acid sequences of a large number (19) of anti-phosophocholine (PC) antibodies isolated from myelomas and hybridomas (Gearhart *et al.*,1981). They found that all V_H regions contained in the IgM class antibody have an identical sequence (prototype) except for the CDR 3 regions, which correspond to the D segments (see below). In contrast, all five V_H regions of the IgG class antibodies contained one to four substitutions. Nine IgA V_H regions fell into two types: five are of the prototype and four were variants. All variant sequences were different. More recently, Kim *et al.* (1981) determined the nucleotide sequences of the two rearranged anti-PC V_H genes and compared them with the nucleotide sequence of the corresponding germline V_H gene that had been sequenced earlier by Crews *et al.* (1981). Bothwell *et al.* also carried out a similar, albeit less extensive, comparison for the anti-NP (nitrophenyl) V_H genes (Bothwell *et al.*, 1981). Results of both of these works confirmed the involvement of somatic mutations in the amplification of the germline-encoded genetic information for Ig V regions.

VII. GENERAL FEATURES OF SOMATIC MUTATIONS IN IMMUNOGLOBULIN GENES

Several interesting features of somatic mutation emerged from all of these studies on the light and heavy chain gene systems. These are:

1. Somatic mutations occur both in CDR's and FR's although they occur about 3.2 and 3.4 times more frequently per base pair in the CDR than in FWR in the light and heavy chains, respectively (Table I).

2. In some genes the base pairs altered by somatic mutations are preferentially purines in the coding strands. Examples are six out of six cases in the unexpressed, but rearranged, κ genes from myeloma T2 (Pech *et al.*, 1981), 4 out of the 4 cases in the MOPC 167 κ gene (Selsing and Storb, 1981; Gerhsenfeld *et al.*, 1981), three out of three cases in the H2020 (Bernard *et al.*, 1978), and S43λ1 genes (A. Bothwell, personal communication)

TABLE I. Somatic Mutations-in Ig Genes–CDR versus FWR

Cahin	No. of genes	Base substitutions			Substitution Density (%)		
		Total	CDR	FWR	CDR	FWR	CDR/ FWR
Light	7	29	15	14	2.65	0.83	3.2
Heavy	5	43	23	20	5.68	1.67	3.4

TABLE II. Somatic Mutations in Ig Genes–Silent versus Replacement

Chain	No. of genes	Base substitutions			
		Total	Silent	Replace.	Silent/Total
Light	7	29	8	21	0.28
Heavy	5	45	15	30	0.33

and 9 out of 10 cases in the S43γ2a gene (Bothwell *et al.*, 1981). However, this purine preference does not apply to all genes studied.

3. Mutations can occur on both copies of the chromosome as long as the genes are rearranged, even if the rearrangement is abnormal in the sense that it does not lead to the synthesis of a functional protein. In two cases studied, unrearranged *V* genes remain unmutated in myelomas (Seidman *et al.*, 1978; Heinrich and Tonegawa, unpublished).

4. Of the total of 74 mutations identified in the translated regions of 12 genes, 23 (32%) mutations are silent (Table II), which is slightly but not very much higher than expected from random changes. This indicates that only a minor degree of selection against replacement changes operates during the immune response.

5. The target of mutations is strikingly restricted to the *V*-coding region and a few hundred base pairs long flanking regions. No mutation, silent or replacing, has ever been found in the *C*-coding regions (Fig. 4).

6. It appears that somatic mutations do not occur, or occur very rarely in the V_H gene segments of IgM class antibodies, while they are abundant in the V_H segments for IgG and IgA. This suggests that the somatic mutations are events associated with peripheral lymphatic systems where μ chain genes undergo "switch recombination" to generate expressed γ, ϵ and α chain genes.

Overall results suggest that lymphocytes have a specific apparatus for introducing mutations in the Ig *V*-coding DNA segments in their neighborhood. This apparatus seems to be activated in the peripheral lymphatic

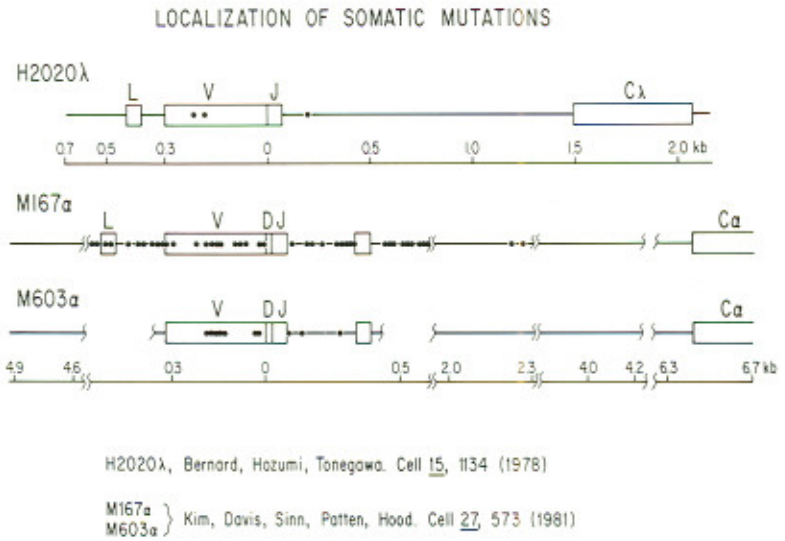


Fig. 4. Localization of somatic mutations. Each filled circle represents the position of a somatic mutation.

organs and acts on the rearranged *V* genes. It constitutes one of the two principal mechanisms by which the Ig genetic information encoded in the germline genome is diversified somatically (see below).

VIII. SOMATIC RECOMBINATIONS OF Ig GENES

The other principal somatic mechanism for Ig gene diversification is recombination (Hozumi and Tonegawa, 1976). It is well established that the *V* regions of the light and heavy chains are encoded in two (Brack *et al.*, 1978; Bernard *et al.*, 1978; Lenhard-Schuller *et al.*, 1978) and three (Sakano *et al.*, 1980; Early *et al.*, 1980) separate gene segments in the germline genome. In the light chains of both κ and λ types, the amino acid residues 1 through 95 or 96 are encoded by the *V* DNA segment while residues 95 or 96 through 108 are encoded by the *J* DNA segment. During development of the B cells in the central lymphatic organ (i.e., bone marrow) one of the multiple copies of the *V* DNA segments and one of the multiple copies of *J* DNA segments join to create a fused *VJ* DNA segment coding for the entire V region. The details of the organization of the two types of gene segments differ somewhat between the κ and λ chain systems. As shown in Fig. 5, the κ complex contains a few hundred different *V* DNA copies and four different *J* DNA copies, the latter being a few kilobases upstream of a single-copy *C* region-coding segment (Sakano *et al.*, 1979; Max

et al., 1980). The somatic recombination occurs between the *V* and *J* DNA segments apparently more or less randomly.

The λ1 light chain was the first Ig gene system in which the segmental nature and somatic rearrangement were established at the nucleotide sequence level (Brack *et al.*, 1978; Bernard *et al.*, 1978; Tonegawa *et al.*, 1977, 1978; Brack and Tonegawa, 1977). Recent studies revealed the organization of the various λ subtype gene sequences. As shown in Fig. 5, four C_λ coding DNA segments each having its own J_λ segment have been identified (Blomberg *et al.*, 1981). $C_{\lambda 1}$, $C_{\lambda 2}$, and $C_{\lambda 3}$ DNA segments correspond to the three mouse light chain subtypes λ1, λ2, and λ3, respectively. The nucleotide sequence suggests that $J_{\lambda 4}$ is defective (Blomberg and Tonegawa, 1982). Two germline V_λ DNA segments, $V_{\lambda 1}$ and $V_{\lambda 2}$, have been identified (Brack *et al.*, 1978). The somatic joinings between the two V_λ segments and three productive J_λ segments seem to occur with some degree of preference: $V_{\lambda 1}$ joins primarily with $J_{\lambda 3}$ or $J_{\lambda 1}$, while $V_{\lambda 2}$ joins primarily with $J_{\lambda 2}$ (Blomberg *et al.*, 1981). However, this preference is not absolute (H. Eisen, personal communication).

In the heavy chains, the amino acid residues 1 to about 94 are encoded in the *V* DNA segments while residues about 100 to 113 are encoded by the *J* DNA segments (Fig. 5). The oligopeptides of varying lengths intervening the *V* and *J* regions are encoded by the third DNA segment referred to as *D* (Sakano *et al.*, 1980; Early *et al.*, 1980; Sakano *et al.*, 1981; Kurosawa *et al.*, 1981). Thus, the formation of a complete V_H gene requires

Fig. 5. Organization of mouse immunoglobulin genes. The exon-intron structure of all heavy chain *C* gene segments except for those of $C\mu$ are abbreviated.

two recombinations, *V-D* joining and *D-J* joining. There exist a few hundred different *V* DNA copies, about twenty different *D* DNA copies (Kurosawa and Tonegawa, 1982), and four different *J* DNA copies (Sakano *et al.*, 1980). As in the κ gene family, *V-D* and *D-J* joining seem to occur with some degree of randomness.

The *V-J* and *V-D-J* joining provide the immune system with two mechanisms by which the germline-encoded genetic information for Ig chains is diversified somatically. The first mechanism, referred to as "combinatorial diversity," is based on the fact that the *V* regions are encoded in two or three types of gene segments each of which exists in multiple copies that are slightly different from each other. Random joining of these gene segments in the population of lymphocytes can create different Ig genes which could be as many as the product of the copy numbers of each gene segment carried in the germline genome. The second mechanism, referred to as "junctional diversity," is based on the fact that the exact joining ends of these gene segments are not uniquely predetermined (Sakano *et al.*, 1979; Max *et al.*, 1980). Thus, the complete genes created by the same pair or set of gene segments can have different codons, or small deletions or insertions of codons, at the joints. Both of these somatic mechanisms operate in the bone marrow or fetal liver where the stem cells develop into Ig-positive cells.

IX. STRUCTURE AND GENETIC ORIGIN OF T CELL ANTIBODY

While the genetic origin of the diversity of Ig's is uncovered in its outlines, the analogous question on the other half of the antibody population, namely T cell receptors, remain totally unresolved. In fact, our knowledge on the chemical structure of these proteins is hopelessly limited. T cell receptors can recognize antigens as exquisitely as Ig's, but there occurs an important difference in the mode of recognitions by Ig's and T cell receptors. Namely, while Ig's recognize and bind antigens by themselves, the T cell receptors recognize only cell-bound antigens in the context of proper gene products encoded in the major histocompatibility complex (MHC) (Fig. 6). This phenomenon, discovered by Zinkernagel and Doherty (1974), is referred to as "MHC restriction." (Some T cells such as a certain type of "suppressor T cells" are not MHC restricted.) The genetic origin of the Ig diversity being virtually identified, the structure and the genetic origin of T cell receptors emerge as the most central issues in immunology. The studies aimed at the solution of these issues will not only by highly relevant for our understanding of the immune system but also have a strong impact on our ability to manipulate the immune responses for the improvement of human health.

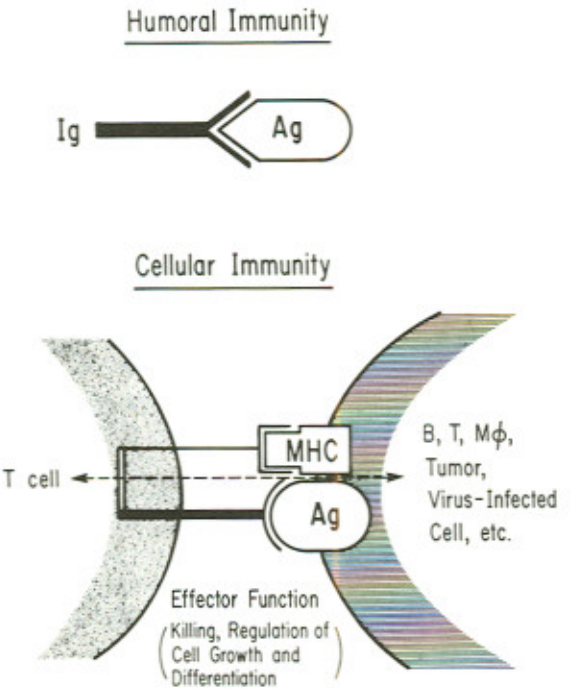


Fig. 6. Comparison of antigen-antibody interactions in humoral and cellular immunity.

X. INITIAL ATTEMPTS TO IDENTIFY T CELL RECEPTOR GENES

Given the fact that the T cell receptors are a minor cellular component and, therefore, have been elusive to the straightforward biochemical approach, the recombinant DNA technology emerges as one of the most hopeful methods. Here the general strategy is to isolate the candidate cDNA clones for the T cell receptor and then prove one of them, indeed, codes for it. Once the cDNA is identified, analysis of the genomic DNA sequences or the gene product (i.e., the receptor) is relatively straightforward. In employing this approach one conservative point at the start is to assume the antigen-recognizing part of the receptor is encoded by an Ig*V* gene itself or by a gene closely related to it structurally or evolutionally. We thus isolated a panel of V_H gene probes and screened a series of antigen-specific killer T cell clones for the occurrence of mRNA [cytoplasmic poly (A)$^+$ RNA] complementary to the probe sequence. As is the case in the V_H sequences (see above), a given V_H gene segment can usually detect from several to a few dozen related germline V_H gene segments when used as a hybridization probe in the Southern gel blot analysis of embryo DNA. The

twelve V_H sequences which we used as probes correspond to the V_H regions classified into twelve different "groups" (Potter, 1977), and the sets of the V_H gene segments detected by these probes are largely nonoverlapping. After screening of seven different T cell clones with some of the V_H probes, we found one combination of the T cell clone and the probe that gave a positive result (see Fig. 7). Here the V_H sequence expressed in a B cell hybridoma B5291 having anti-NP specificity detected an RNA band at about 2.7 kb in the total cytoplasmic poly $(A)^+$ RNA prepared from a cytolytic T cell clone, DFL 12, specific to fluorescein and with an $H\text{-}2^d$ haplotype. The occurrence of the RNA band is specific in the sense that six other functional T cell clones did not show any positive band with the B5291 V_H probe and that seven other V_H probes tested did not give a positive band in DFL 12 RNA.

We have recently cloned the cDNA transcript of the 2.7 kb mRNA in a derivative of pBR322. We plan to characterize the cDNA clone by a variety of methods including DNA sequencing. We will use the cDNA clone in a

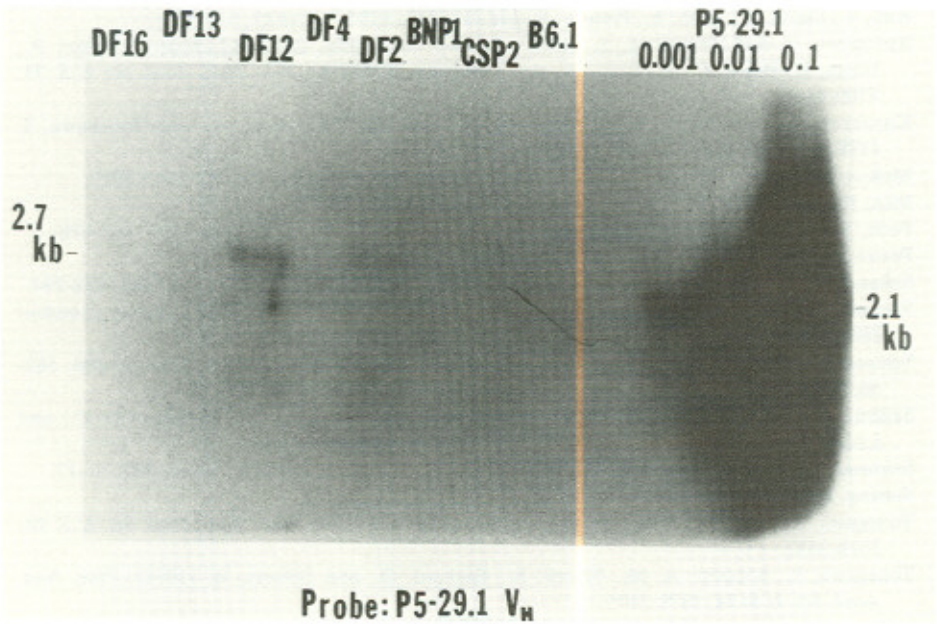


Fig. 7. Detection of a specific poly $(A)^+$ RNA in a cytolytic T cell clone DFL. 12 with a V_H probe P5-29.1. Ten micrograms each of cytoplasmic poly $(A)^+$ RNA from eight different cytolytic T cell clones were analyzed by the "Northern gel blot" method using a nick-translated V_H probe isolated from a hybridoma P5-29.1. In the rightmost three channels, indicated amounts (in micrograms) of partially purified heavy chain mRNA from hybridoma P5-19.1 were hybridized as positive controls.

variety of experiments in order to test the hypothesis that it codes for at least part of the recognition molecule on the surface of the cytolytic T cells. We hope that these studies will not only decipher the structure of T cell receptors, but also will help us to uncover the molecular mechanism underlying T-B collaboration.

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