Structural Heterogeneity and Functional Domains of Murine Immunoglobulin G Fc Receptors

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Binding of antibodies to effector cells by way of receptors to their constant regions (Fc receptors) is central to the pathway that leads to clearance of antigens by the immune system. The structure and function of this important class of receptors on immune cells is addressed through the molecular characterization of Fc receptors (FcR) specific for the murine immunoglobulin G isotype. Structural diversity is encoded by two genes that by alternative splicing result in expression of molecules with highly conserved extracellular domains and different transmembrane and intracytoplasmic domains. The proteins encoded by these genes are members of the immunoglobulin supergene family, most homologous to the major histocompatibility complex molecule E_β. Functional reconstitution of ligand binding by transfection of individual FcR genes demonstrates that the requirements for ligand binding are encoded in a single gene. These studies demonstrate the molecular basis for the functional heterogeneity of FcR's, accounting for the possible transduction of different signals in response to a single ligand.

FOR THE IMMUNE SYSTEM TO ACHIEVE ITS GOAL OF PROTECTION of the organism against foreign antigens, cooperation between the humoral and cellular pathways occurs with the interaction of antibody-antigen complexes with effector cells, mediated by specific antibody receptors, Fc receptors (FcR). Receptors for the Fc domain of immunoglobulin G (FcγR) are present on B cells, T cells, NK cells, macrophages, and polymorphonuclear leukocytes (*1–3*). The binding of immune complexes to FcγR's on neutrophils and macrophages triggers various cellular responses, including phagocytosis, release of activated oxygen metabolites and mediators of inflammation such as leukotrienes and prostaglandins, and induction of neutral hydrolases (*4*). The FcγR's have also been described on lymphocytes (*5*) where they are presumed to have a role in the modulation of antibody production by B cells.

Although FcR's have been described for all classes of immunoglobulin (*1–3*), little is known regarding their structure. Among the best characterized FcR's are the high avidity basophil and mast cell immunoglobulin E (IgE) receptor (FcεR) and the mouse macrophage FcR that binds IgG2b and IgG1 immune complexes (Fcγ2b/γ1R). Studies with murine macrophages have demonstrated independent binding sites for IgG3 (Fcγ3R) and IgG2a (Fcγ2aR), and IgG2b/IgG1 complexes (Fcγ2b/γ1R) (*6–9*). The study of the latter receptor has been facilitated by the isolation of a monoclonal antibody (mAb), which is called 2.4G2, and which is directed against an epitope present on murine FcγR's of both macrophages and lymphocytes (*10*). The macrophage Fcγ2b/γ1R has been characterized as an integral membrane, 50- to 60-kD glycoprotein with four sites for N-linked glycosylation (*11*).

Recently it has been shown (*12–14*) that the isotype specificity of the lymphocyte receptor reactive with mAb 2.4G2 is broader than the macrophage Fcγ2b/γ1R, which does not bind IgG2a. In addition, the Fcγ2b/γ1R has been identified as an alloantigen related to the Mls system (*15, 16*), a locus on chromosome 1 that specifies products on antigen presenting cells and governs an H-2 compatible T cell proliferative response resulting in intense stimulation of the mixed lymphocyte reaction. Four alleles of this locus have been identified (*17*). The Ly-17 antigen, which is identical to Fcγ2b/γ1R, is either closely linked to or determined by this locus (*18*).

In order to define the structural characteristics of these receptors, account for the differences in isotype specificity between macrophages and lymphocytes, and characterize the basis for their functional heterogeneity, we have isolated and characterized complementary DNA's (cDNA's) from macrophage and T cell lines that encode these receptors and demonstrate their ability to function as FcγR's upon transfection. We have identified two genes that encode proteins with highly homologous extracellular domains capable of binding immunoglobulin but have different transmembrane and cytoplasmic domains. One is expressed only in macrophages, whereas the other is expressed in both macrophages and lymphocytes. Coupled to this heterogeneity is the observation that alternative splicing may give rise to alternative cytoplasmic domains, further extending the functional diversity of the FcγR in a cell type–specific fashion. Finally, the structure of the FcγR suggests that it is a member of the immunoglobulin supergene family (*19*) most homologous to the major histocompatibility complex (MHC) class II molecule E_β.

Isolation and sequence analysis of a macrophage FcγR cDNA. The amino terminal 22 amino acids for the S49.1 Fcγ2b/γ1R were

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sequenced on 200 pmol of protein purified by conventional methods (*20–22*) with a gas phase microsequencing apparatus (Applied Biosystems, model 470A) and found to be THDLPKAVVKLEPPWIQVLKED (*23*). The seven amino acids EPPWIQV were chosen for the synthesis of a corresponding oligodeoxynucleotide based on their relatively low degeneracy. After considering preferred codon usage (*22*) and the ability of G-T base pairing to form, we synthesized a 20-nucleotide sequence that corresponds to the complementary strand of the sequence encoded above. That sequence is 5′ ACTTG$\overset{G}{T}$ATCCA$\overset{G}{T}$GG$\overset{G}{T}$GGTTC 3′. This probe was end-labeled to high specific activity with [γ-^{32}P]ATP (adenosine triphosphate) and used to screen a mouse macrophage cDNA library constructed to size-fractionated J774 messenger RNA (mRNA) in the plasmid vector pUC-9 (*24*). A library of 50,000 clones was screened from which two positives were identified. The clone with the largest insert was chosen for subsequent analysis and called FcγRα (Fig. 1, A and B). An open reading frame of 782 nucleotides was found, beginning with an ATG at position 64 and terminating at position 846. The predicted signal peptidase cleavage site was assigned on the basis of the consensus rules for such sequences (*25*). The 19–amino acid sequence, encoded from nucleotides 160 to 210, is identical to amino acids 3 to 22 of the S49.1 sequence presented above, with the exception of position 12, in which a glutamic acid residue in S49.1 is replaced by an aspartic acid in J774. The difference in the first three amino acids and position 12 resulted from the heterogeneity between the macrophage and T cell proteins (see below). The deduced protein sequence contains two regions of hydrophobic amino acid residues (Fig. 1B). These regions encode the putative signal sequence (nucleotides 64 to 153) and a transmembrane anchor sequence (nucleotides 709 to 768). An extracellular domain of 185 amino acids is expected for the mature protein, which contains four potential N-linked glycosylation sites (Fig. 1B) as well as four cysteine residues (Fig. 1B) that could form two intrachain disulfide bonds. A serine- and threonine-rich region is encoded from amino acids 155 to 185, just preceding the transmembrane domain, in which 30 percent of the residues are represented by these two amino acids. An intracytoplasmic domain of 26 amino acids is predicted from this sequence. The primary sequence predicts a molecular mass of 30,040 daltons, which would then be subject to glycosylation at the four N-linked sites described, and possibly O-linked sites as well.

This extracellular domain consists of two internally repeated sequences, as indicated by the self-comparison dot matrix analysis (Fig. 2A). Amino acids 10 to 100 of the predicted mature protein show homology to amino acids 101 to 183. A 28.6 percent identity is found over a 63–amino acid overlap. Allowing for similarities based on related side chains, this homology increases to 75 percent. These homologies suggest a structural repeating domain, bounded by the cysteine residues. Database comparison (*26*) of this sequence revealed significant homology to immunoglobulin molecules, MHC class I and II proteins, β_2 microglobulin, and other members of this supergene family. The homology of the extracellular domain to a kappa chain variable (V_κ) region shows clusters of homology centered about the cysteine residues in both protein (Fig. 2B). The

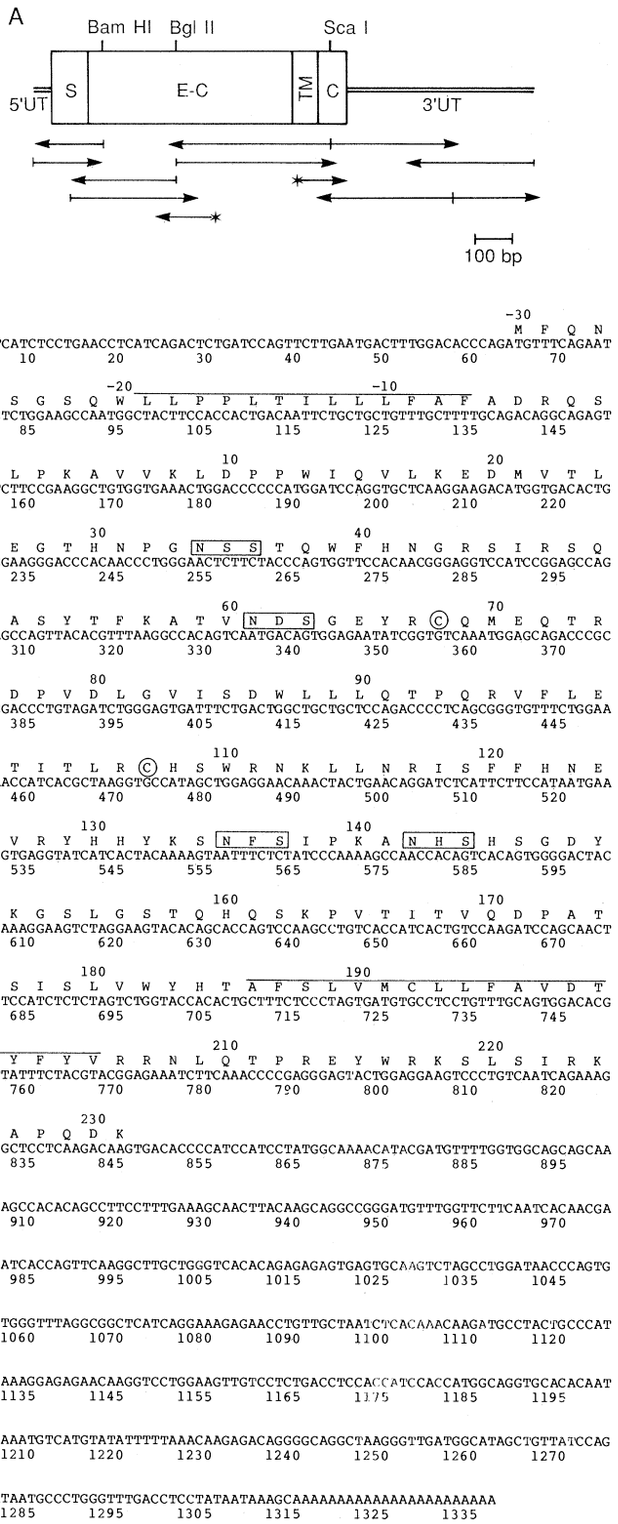


Fig. 1. Restriction map, sequencing strategy, and nucleotide sequence for the macrophage FcγRα. (A) Scheme of the cDNA encoding the α gene: open rectangle indicates the predicted protein coding region and the double lines the 5′ and 3′ untranslated sequences (UT). The coding region is divided into a proposed signal sequence (S), extracellular domain (E-C), transmembrane domain (TM), and a cytoplasmic domain (C). Representative restriction sites are indicated. Arrows indicated the DNA sequences determined: those with blunt tail mark segments determined by the chain termination method (*37*), those arrows with asterisks mark segments determined by chemical degradation (*38*). Repeated determination of the same sequences are not shown. The clone described above was isolated by screening a J774 size-fractionated cDNA library (*24*) to an RNA fraction previously determined to include the mRNA for the FcγR recognized by mAb 2.4G2 (*39*). (B) The translated sequence is presented in the one letter code above the determined nucleotide sequence (*23*). A 30–amino acid signal sequence is predicted, numbered −30 to −1 with a hydrophobic core overlined. The predicted signal peptidase cleavage site is indicated by the arrow between −1 and 1. The N-linked glycosylation sites are boxed, and cysteine residues are circled. A hydrophobic stretch of 20 amino acids presumed to span the membrane is overlined.

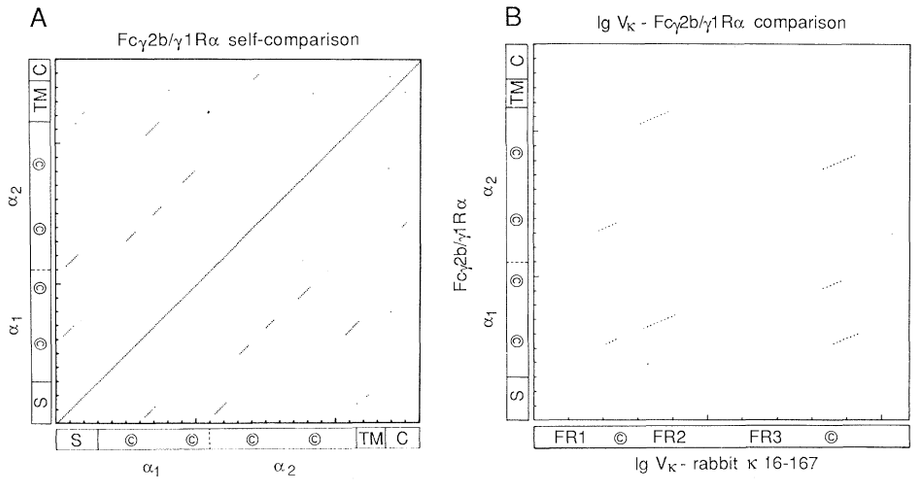


Fig. 2. Immunoglobulin-like structure for the extracellular domain of the FcγRα protein. (A) A self-comparison of the amino acid sequences of the protein presented in Fig. 1B is shown; the DIAGON (*40*) program was used. A line of identity is seen as well as off-diagonal lines, indicative of internally repetitive sequences. A schematic of the proposed domain structure is presented along each axis, with the cysteine residues circled. (B) Comparison of a rabbit V_κ light chain and the FcγRα sequence. The V region sequence is displayed along the abscissa, with the framework regions (FR) indicated, along with the two cysteine residues. The FcγRα sequence is displayed along the ordinate, with the proposed domain structure indicated.

amino acids conserved between each domain of the FcγRα protein are, in general, positions which are invariant residues of all κ light chains. For example, the highly conserved V region sequence Asp-X-Gly-X-Tyr-X-Cys is found at positions 62 to 68 and 145 to 151 of the FcγRα sequence. Similarly, the invariant Ile at position 75 of V regions is found in an analogous position (position 138) of the predicted mature FcγRα sequence. These homologies suggest that the FγcRα sequence consists of two immunoglobulin-like domains, each consisting of a potential disulfide loop of 42 amino acids within a domain of 70 to 80 amino acids.

The most significant homology found for this FcR is to the MHC class II protein E_β, with 32 percent identity over a 91 amino acid region (Fig. 3A). Random shuffling of these two sequences, by means of the program RDF (*27*), indicated that the optimized alignment (Fig. 3A) is highly significant, at five standard deviations (5 SD) above the mean. This homology to E_β occurs in the β_2 domain (Fig. 3B) which itself is an immunoglobulin-like domain. Homology is also apparent by this analysis in the transmembrane domains of these two proteins.

Cell-type specific expression of the macrophage FcγRα. Messenger RNA extracted from a variety of cell lines was analyzed for the presence of message corresponding to the FcγRα sequence. A broad band of hybridization was detected in the macrophage lines P388D1, WEHI 3A, RAW 264.7 and J774 (Fig. 4A), while a T cell line S49.1 contained a higher molecular weight mRNA species. The P388 line showed two RNA species in equal amounts, while WEHI 3A had a major band that migrated faster than 18*S* and a minor band migrating slower than 18*S*. The 2.4G2 negative lines CL.7 (fibroblast), L cell (fibroblast), and L5178Y (T cell) do not contain an α-gene transcript. The cell type specificity of expression of the α gene was apparent when a probe was constructed to the 5′ sequences of the cDNA and used to examine the same macrophage and T cell RNA's (Fig. 4B). No transcript is detected in the S49.1 cell line with this probe, and only a single species was seen in P388. Similarly, the lower abundance species migrating slower than 18*S* in WEHI 3A was not detected with this probe. The T cell line S49.1 and the macrophage-like lines P388 and WEHI 3A therefore contained RNA species cross-hybridizing to the FcγRα gene and differing from the α-transcript. Two other T cell lines (EL-4 and K-36) were also analyzed with the same result as found for S49.1. To identify those transcripts and the genetic basis for their expression, we constructed a cDNA library to size-fractionated S49.1 mRNA and probed with the complete α cDNA probe. Positive clones were identified at a frequency of 1 in 1000 and were characterized by restriction mapping and DNA sequence analysis.

A single open reading frame was found in the S49.1 transcript, called β_1, beginning with an ATG at nucleotide 340 and terminating at nucleotide 1326 (Fig. 5, A and B). The predicted molecular mass of this protein before modification is 36,750 daltons. Beginning at nucleotide 427 (position +1), 22 amino acids are found which are identical to the determined amino terminal sequence for the purified FcγR protein. The sequence that precedes this amino terminus encodes a signal sequence with a characteristic hydrophobic core, which bears no homology to the signal sequence of the α transcript (Fig. 1B). The extracellular domain has 95 percent identity with the α gene sequence (Fig. 6), beginning at amino acid 4 of the β_1 sequence and continuing through amino acid 174. A transmembrane domain and intracytoplasmic region are predicted for the β_1

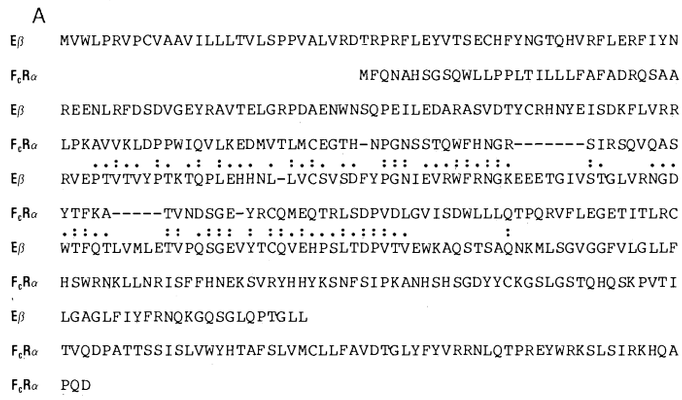
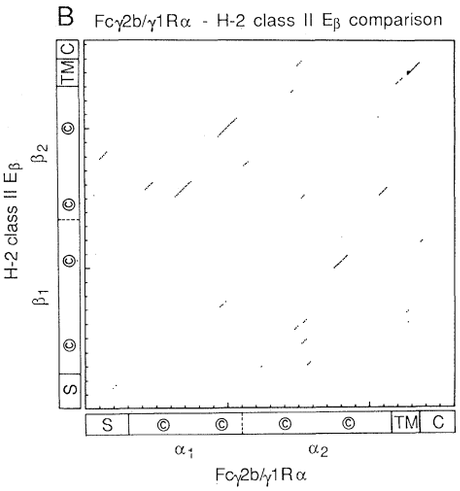


Fig. 3. Homology of the FcγRα to the MHC class II protein E_β. (A) Amino acid alignment of the E_β sequence and the FcγRα sequence; the search program fastp was used (*27*). Two dots indicate identity and one dot indicates a conservative mutation. Gaps have been introduced to obtain the optimized alignment. (B) DIAGON alignment of the sequences displayed above. The domain structure of the two proteins is indicated along each axis.



sequence and these bear no homology to the analogous domains of the α gene sequence. No sequence homology is found in the 5′ and 3′ untranslated domains of these two transcripts.

Four other tryptic peptides were sequenced for the S49.1 FcγR. A sequence of ten amino acids, SQVQASYTFK, was confirmed at position 50 to 59 of the β_1 sequence; the sequence of eight amino acids, ISFFHNEK, was confirmed at positions 120 to 127; the sequence of 13 amino acids, EMGETLPEEVGEY, was present at positions 222 to 235, and finally the 13 amino acid sequence TEAENTITYSLLK was confirmed at positions 271 to 283.

Since both α and β_1 sequences were obtained from cell lines derived from BALB/c mice, the 5 percent sequence variation in the highly conserved extracellular domain could not arise from allelic variation. To confirm that the β_1 transcript was derived from a second gene, Southern blot analysis of DNA obtained from different inbred strains of mice was used to map the α and β genomic sequences. A restriction fragment length polymorphism (RFLP) was detected with an α, but not a β, sequence probe with the enzymes Bgl II and Taq I (Fig. 7A). The complete α and β_1 probes contain highly conserved extracellular domains and cross-hybridize, detecting 4.0-kb, 1.0-kb, and 0.7-kb fragments. However, the RFLP detected with the complete α probe is replaced by a non-polymorphic 2.4-kb band when the complete β probe is used (Fig. 7B). This RFLP maps to the 3′ sequences of the α probe, as determined by reprobing these blots with 3′ single-copy sequence probes derived from the α and β transmembrane and cytoplasmic domains. As is described below, a related β transcript, isolated from

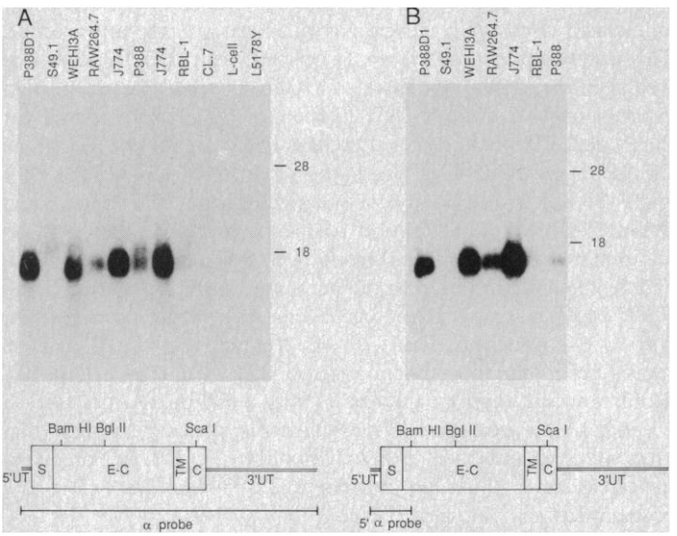


Fig. 4. Distribution of FcγRα transcripts. Macrophage cell lines P388D1 (*41*), WEHI 3A (*42*), RAW 264.7 (*43*) and J774 (*44*) were maintained in culture (*24*). The T cell lines S49.1 (*45*) and L5178Y, the macrophage-like line P388, the rat basophil line RBL-1, the fibroblast lines CL.7 (*45*), and L cells were also maintained in culture (*24*). RNA was isolated by the guanidine isothiocyanate procedure and poly(A)-selected (*46*). Poly(A^+) RNA (1 μg) was fractionated on agarose-formaldehyde gels (*47*), transferred to nitrocellulose, and hybridized under stringent conditions either with the complete α probe (A) or a probe constructed to the 5′ α sequences (B). The ribosomal RNA markers are indicated. Hybridization to the rat basophil line RBL-1 suggests the expression of a related transcript in that cell line, which is positive for both FcεR and FcγR.

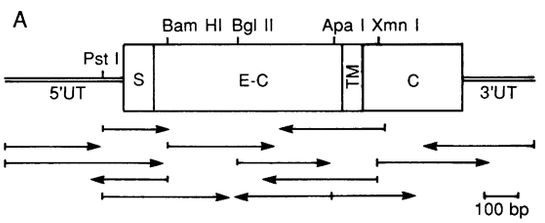


Fig. 5. Restriction map and sequencing strategy (A, above) and nucleotide sequence (B, right) of the T cell FcγRβ_1 cDNA. (A) The restriction map and sequencing strategy for the β_1 cDNA; conventions are as indicated for the legend to Fig. 1A. (B) Nucleotide sequence and predicted amino acid translation for the β_1 cDNA. A signal sequence of 29 amino acids is present with the mature amino terminus indicated by the arrow at position −1, 1. The N-linked glycosylation sites are indicated, as are the cysteine residues. A transmembrane domain of 26 amino acids is overlined. The upward arrows indicate the position of a 138-nucleotide insertion found in this sequence when compared to an otherwise identical sequence (β_2) isolated from the macrophage line J774 (see Fig. 9B).

B

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AATGTATGTGAAGGATGAGTGTTCTACTGCTGTTCTCACATATGGCCTAGCTTTTGGTCTACAATCCAACAGTGA
        10        20        30        40        50        60        70

GCTGGGGATTGTGATAATAGAGAAACCCAGATGGTGACTGAGGCAAATGACTTCTGAGCTGCGTTGGGGTGAAGT
        85        95       105       115       125       135       145

TTTCCCTCTCTGTACCAGACGTCCAGGTCAGCTACGGCTCCAGCAGAACATGAAGGGAGTTGTTTCTCAGTGTCT
       160       170       180       190       200       210       220

AAAAAATTTTCTGTGATTTGAGCTGAATCCAGTTTATTCTGCCGGGAGGAAGCCTGTGCCTGCAGCTGACTCGCT
       235       245       255       265       275       285       295

                                      -29                 -20
                                       M  E  S  N  W  T  V  H  V  F  S  R
CCAGAGCTGATGGGAATCCTGCCGTTCCTACTGATCCCCATGGAGAGCAACTGGACTGTCCATGTGTTCTCACGG
       310       320       330       340       350       360       370

                -10                    -1 1
 T  L  C  H  M  L  L  L  W  T  A  V  L  N  L  A  A  G  T  H  D  L  P  K  A  V
ACTTTGTGCCATATGCTACTGTGGACAGCCGTGCTAAATCTTGCTGCTGGGACTCATGATCTTCCAAAGGCTGTG
       385       395       405       415       425       435       445

   10                        20                        30
 V  K  L  E  P  P  W  I  Q  V  L  K  E  D  T  V  T  L  T  Ⓒ  E  G  T  H  N
GTCAAACTCGAGCCCCCGTGGATCCAGGTGCTCAAGGAAGACACGGTGACACTGACATGCGAAGGGACCCACAAC
       460       470       480       490       500       510       520

                40                        50
 P  G  N  S  S  T  Q  W  F  H  N  G  R  S  I  R  S  Q  V  Q  A  S  Y  T  F
CCTGGGAACTCTTCTACCCAGTGGTTCCACAATGGGAGGTCCATCCGGAGCCAGGTCCAAGCCAGCTACACGTTT
       535       545       555       565       575       585       595

   60                        70                        80
 K  A  T  V  N  D  S  G  E  Y  R  Ⓒ  Q  M  E  Q  T  R  L  S  D  P  V  D  L
AAGGCCACAGTCAATGACAGTGGAGAATATCGGTGTCAAATGGAGCAGACCCGCCTCAGCGACCCTGTAGATCTG
       610       620       630       640       650       660       670

                90                       100
 G  V  I  S  D  W  L  L  L  Q  T  P  Q  L  V  F  L  E  G  E  T  I  T  L  R
GGAGTGATTTCTGACTGGCTGCTGCTCCAGACCCCTCAGCTGGTGTTTCTGGAAGGGGAAACCATCACGCTAAGG
       685       695       705       715       725       735       745

  110                       120                       130
Ⓒ  H  S  W  R  N  K  L  L  N  R  I  S  F  F  H  N  E  K  S  V  R  Y  H  H
TGCCATAGCTGGAGGAACAAACTACTGAACAGGATCTCGTTCTTCCATAATGAAAAATCCGTGAGGTATCATCAC
       760       770       780       790       800       810       820

                140                       150
 Y  S  S  N  F  S  I  P  K  A  N  H  S  H  S  G  D  Y  Y  Ⓒ  K  G  S  L  G
TACAGTAGTAATTTCTCTATCCCAAAAGCCAACCACAGTCACAGTGGGGACTACTACTGCAAAGGAAGTCTAGGA
       835       845       855       865       875       885       895

  160                       170                       180
 R  T  L  H  Q  S  K  P  V  T  I  T  V  Q  G  P  K  S  S  R  S  L  P  V  L
AGGACACTGCACCAGTCCAAGCCTGTCACCATCACTGTCCAAGGGCCCAAGTCCAGCAGGTCTTTACCAGTATTG
       910       920       930       940       950       960       970

                190                       200
 T  I  V  A  A  V  T  G  I  A  V  A  A  I  V  I  I  L  V  S  L  V  Y  L  K
ACAATTGTGGCTGCTGTCACTGGGATTGCTGTCGCAGCCATTGTTATTATCCTAGTATCCTTGGTCTATCTCAAG
       985       995      1005      1015      1025      1035      1045

  210                       220                       230
 K  K  Q  V  P  A  L  P  G  N  P  D  H  R  E  M  G  E  T  L  P  E  E  V  G
AAAAAGCAGGTTCCAGCTCTCCCAGGAAACCCTGATCACAGGGAAATGGGAGAAACCCTTCCAGAGGAAGTAGGT
      1060      1070      1080      1090      1100      1110      1120

                240                       250
 E  Y  R  Q  P  S  G  L  S  A  C  Q  P  R  A  P  S  G  L  E  P  T  S  S  S
GAGTACAGACAACCCTCTGGGCTCAGTGCCTGTCAGCCCAGGGCTCCATCTGGACTGGAGCCAACAAGCAGCAGC
      1135      1145      1155      1165      1175      1185      1195

  260                       270                       280
 P  Y  N  P  P  D  L  E  E  A  A  K  T  E  A  E  N  T  I  T  Y  S  L  L  K
CCATACAATCCTCCTGATCTGGAAGAAGCTGCCAAAACTGAGGCTGAGAATACGATCACCTACTCACTTCTCAAG
      1210      1220      1230      1240      1250      1260      1270

                290                       300
 H  P  E  A  L  D  E  E  T  E  H  D  Y  Q  N  H  I
CATCCCGAAGCCCTGGATGAAGAAACAGAGCATGATTACCAGAACCACATTTAGTCTCCCTTGGCATTGGGAAAA
      1285      1295      1305      1315      1325      1335      1345

GCAAGCCAGAAAGGCCAGGATCTAGTGTCTCCTGGTCCAAGGGATGCTGTAGATATTAAAGAAAACATCCAGAGT
      1360      1370      1380      1390      1400      1410      1420

CACTTCTGTGAGTCCTGAAACCAACAGACACTACGAGATTGGTTCCCAATGGTTGACTGTACTAATGACTCCCAT
      1435      1445      1455      1465      1475      1485      1495

AACTTACAGCTTCCCAACTCAAGACTCTTCTGCTATCGATCCACACTGCCACTAAAATTAATCAACTTACTGCC
      1510      1520      1530      1540      1550      1560      1570
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FcRβ1  MESNWTVHVFSRTLCHMLLWTAVLNLAAGTHDLPKAVVKLEPPWIQVLKEDTVTLTCEGT
                                  :::::::::.:::::::::::: ::: ::::
FcRα   MFQNAHSGSQWLLPPLTILLLFAFADRQSAALPKAVVKLDPPWIQVLKEDMVTLMCEGT

FcRβ1  HNPGNSSTQWFHNGRSIRSQVQASYTFKATVNDSGEYRCQMEQTRLSDPVDLGVISDWLL
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
FcRα   HNPGNSSTQWFHNGRSIRSQVQASYTFKATVNDSGEYRCQMEQTRLSDPVDLGVISDWLL

FcRβ1  LQTPQLVFLEGETITLRCHSWRNKLLNRISFPHNEKSVRYHHYSSNFSIPKANHSHSGDY
       ::::: ::::::::::::::::::::::::::::::::::.:::::::::::::::::
FcRα   LQTPQRVFLEGETITLRCHSWRNKLLNRISFPHNEKSVRYHHYKSNFSIPKANHSHSGDY

FcRβ1  YCKGSLGRTLHQSKPVTITVQGPKSSRSLPVLTIVAAVTGIAVAAIVIILVSLVYLKKKQ
       ::::::::.: :::::::::::.:
FcRα   YCKGSLGSTQHQSKPVTITVQDPATTSSISLVWYHTAFSLVMCLLFAVDTGLYFYVRRNL

FcRβ1  VPALPGNPDHREMGETLPEEVGEYRQPSGLSACQPRAPSGLEPTSSSPYNPPDLEEAAKT

FcRα   QTPREYWRKSLSIRKHQAPQD

FcRβ1  EAENTITYSLLKHPEALDEETEHDYQNH
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Fig. 6. Amino acid alignment of the FcγRα and FcγRβ$_1$ sequences. Alignment of these two FcγR sequences was obtained by the fastp (*27*) analysis. Eight amino acid differences are found over the extracellular domain, for an overall 95 percent identity.

the same macrophage line (J774) has precisely the same β$_1$ extracellular domain sequence, further demonstrating that the 5 percent sequence variation between α and β transcripts cannot result from cell line variation.

Expression of the β gene in T cell and macrophage lines. The β$_1$ cDNA probe was used to analyze the expression of this gene in various cell lines (Fig. 8, A and B). This probe detected the S49.1 transcript, and cross-hybridized to detect the α transcript in macrophage lines. However, comparison of the results of this blot with that in Fig. 4A indicates that a different pattern of transcripts is present, differing with respect to amounts of particular mRNA's. In particular WEHI 3A and P388 demonstrate two transcripts of comparable abundance, one of which has the same apparent mobility as an S49.1 transcript. This is confirmed with the use of a 5′ β$_1$ probe (Fig. 8B), constructed to the 5′ untranslated region of this transcript in which no homology to the α gene is found. Transcripts are detected in macrophage lines with this probe, demonstrating that the β gene is expressed in macrophages, resulting in a different transcript size in J774, RAW 264.7, and P388D1 lines compared to that found in the S49.1 T cell line. To investigate these macrophage β transcripts, we screened the J774 cDNA library with probes derived from the β gene. Restriction enzyme mapping of the clones obtained demonstrated that these clones differed from the β$_1$ transcript of the S49.1 cell line in lacking an Xmn I site (Fig. 9, A and B).

The β transcript isolated from J774, called β$_2$, is identical throughout its length to the β$_1$ sequence, both for coding and noncoding sequences. However, a 138-nucleotide insertion is found in the β$_1$ sequence, nucleotides 1066 to 1204 (Fig. 5b), which occurs after nucleotide 783 in the β$_2$ sequence and results in a 46–amino acid insertion in the cytoplasmic domain of the β$_1$ transcript. This insertion accounts for the larger transcript found in S49.1 as well as in the T cell lines EL-4 and K-36. Ribonuclease protection experiments with an [α-^{32}P]CTP generated RNA probe specific for the β$_1$ gene detected protected fragments specific for the β$_1$ gene in the S49.1 and K-36 T cell lines, the 93-4 pre-B cell line, the Bcl-I and A-20 B cell lines, and not in the macrophage lines P388D1 and RAW 264.7 (*28*). In addition to the β$_1$ protected fragment, an additional fragment was detected in both S49.1, K-36, 93-4, Bcl-I, and A-20, and this fragment comigrated with the P388D1 and RAW 264.7 macrophage protected fragment, with a size consistent with a β$_2$ transcript. These results suggest that the β gene is transcribed in lymphocytes and macrophages. Sequence analysis of a T cell, however, revealed a transcript with a 138-bp insertion, which is most simply explained by an alternative splicing pathway that gives rise to an additional exon in lymphocyte FcγRβ. The predicted molecular mass of the β$_2$ protein is 31,886 daltons before any posttranslational modification.

Functional domains of the FcγR: Expression in transfected cell lines. In order to begin to assess the functional role of the structural heterogeneity described for this FcR and to ascertain if more than one polypeptide chain was necessary for ligand binding, we transfected FcR negative cell lines with the β cDNA clones. Expression was achieved by cloning the coding sequence of the β$_1$ or β$_2$ cDNA's into an expression vector (pcEXV-3) (*29*) in which the SV40 early promoter is used to achieve transcription of the cloned

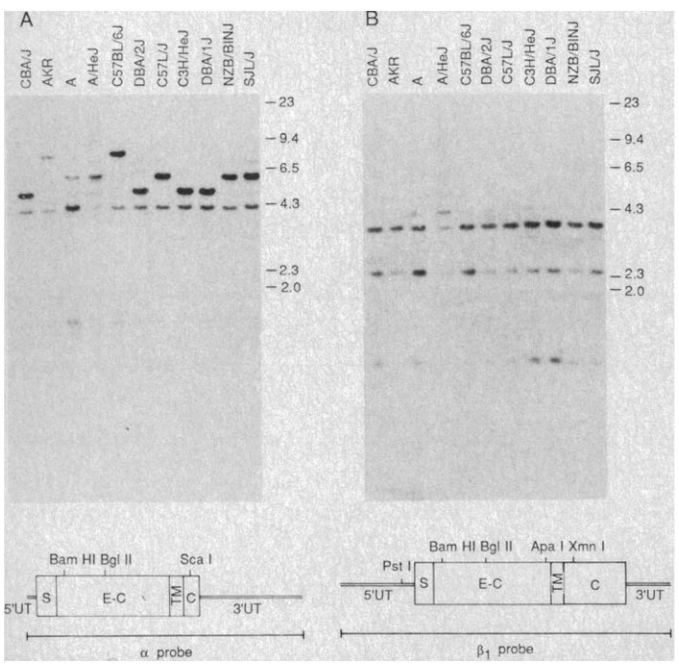

Fig. 7. RFLP analysis of the FcγR α and β genes. Liver DNA was isolated from the strains indicated, subjected to Taq I restriction endonuclease digestion, and analyzed by Southern blot hybridization with the DNA fragments indicated, under conditions of high strigency. The inbred strains were chosen on the basis of their Mls allele: AKR, NZB/BINJ, DBA/2J, DBA/1J "a"; C57BL/6J, C57L/J, BALB/c "b"; A, SJL/J, A/HeJ, C3H/HeJ "c"; and CBA/J "d". DNA samples were digested and placed onto two parallel gels. Molecular size markers are indicated.

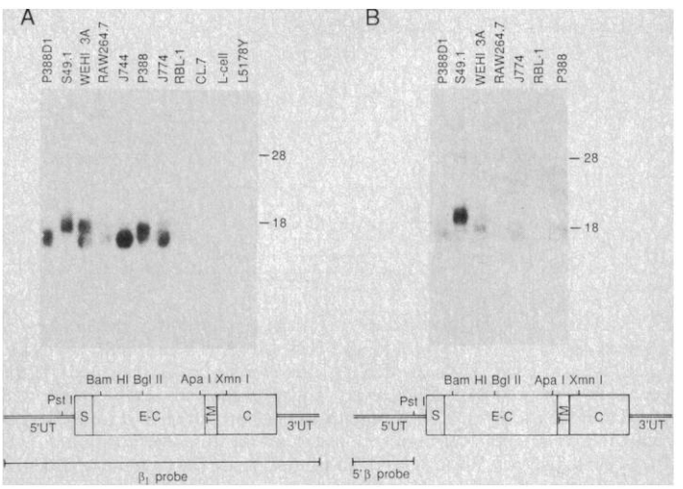

Fig. 8. Distribution of FcγRβ$_1$ transcripts. RNA blots prepared as described in the legend to Fig. 4 were probed either with the full-length β$_1$ probe (A) or a probe constructed to the 5′ untranslated region of β$_1$ (B).

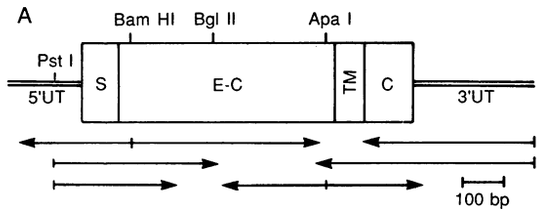

Fig. 9. Restriction map and sequencing strategy and nucleotide sequence of the macrophage FcγRβ$_2$ cDNA. (A) The physical map and proposed domain structure for the β$_2$ cDNA is presented along with the sequencing strategy, according to the convention set forth in the legend to Fig. 1A. Representative restriction sites are indicated. (B) Nucleotide sequence and predicted amino acid sequence for the β$_2$ cDNA sequence.

sequences. Mouse melanoma cells (B78H1) were cotransfected with the plasmid constructions and pGCcos3neo, which confers resistance to the drug G418 (*30*). After 10 days in G418-containing medium colonies were screened by rosetting with human erythrocytes conjugated with the monoclonal antibody 2.4G2 (*31*). Positive cells were cloned and then tested for FcγR activity.

Stable lines expressing the 2.4G2 epitope avidly bound sheep red blood cells (sRBC) opsonized with rabbit antiserum to sRBC, diagnostic of FcγR function (Fig. 10A). In addition, this binding is blocked in a concentration dependent fashion by mAb 2.4G2 (Fig. 10, B, C, and D), demonstrating the specificity of binding to this receptor. Transfectants that were obtained with the β$_1$ insert in the expression vector demonstrated the same pattern of binding. Controls with untransfected B78H1 cells transfected cells in which the FcγRβ sequences were in the reverse orientation with respect to the SV40 promoter, and studies with sRBC's not coated with antibody all gave negative results. These experiments indicate that the protein expressed by these cDNA clones is able to be displayed on the cell surface and mediate the binding of antibody-antigen complexes in a specific manner. Experiments with other cell lines, such as mouse L cells or monkey COS cells suggest that the ability of these sequences to specify an FcR is not cell type–specific.

The FcR's link the humoral and cellular immune systems by serving to translate the exquisite specificity of antigen recognition of

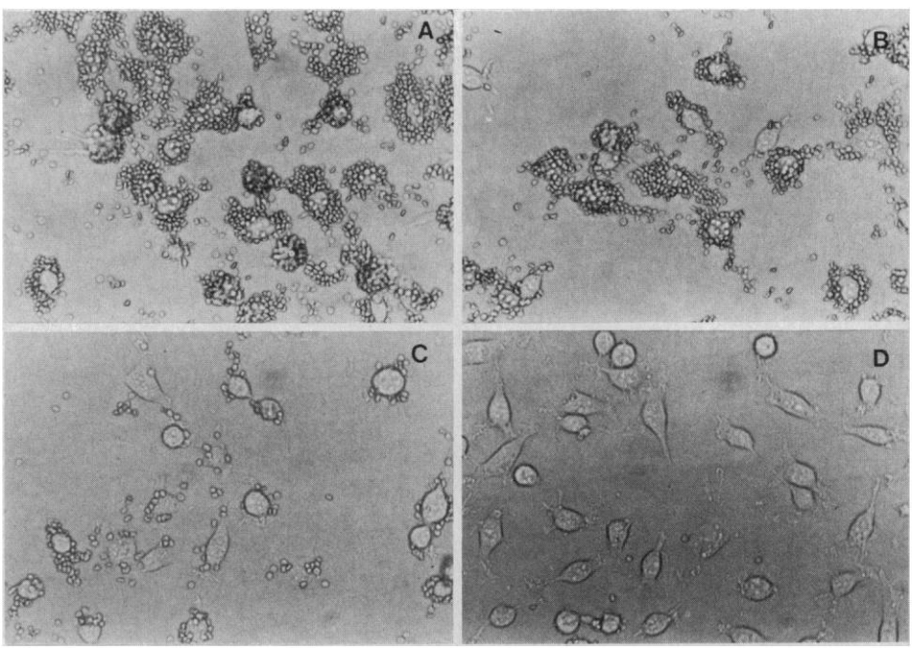

Fig. 10. Reconstitution of immunoglobulin binding activity by FcγRβ$_2$ transfection. The 1100-bp Pst I fragment derived from the β$_2$ clone (Fig. 9) was cloned into the Sma I site of the eukaryotic expression vector pcEXV-3. The recombinant pcEXV-3β$_2$ (500 ng) was cotransfected with 150 ng of pGCcos3neo and 19 μg of mouse melanoma DNA of large molecular size (*48*) onto B78H1 mouse melanoma cells by the calcium phosphate precipitation technique (*49*) and were subjected to geneticin (G418, Gibco Laboratories) selection for 10 days. Transfectants were screened with 2.4G2 conjugated human O erythrocytes (*50*) and positive rosettes were scored. Clonal lines of the 2.4G2 positive transfectants were tested for their ability to rosette IgG coated sRBC prepared with rabbit antiserum to sRBC (Cappel) and sRBC (GIBCO) at 1:12,000 nonagglutinating titer of antibody. The specificity of receptor expression was determined by inhibition with mAb 2.4G2. The transfectants were incubated at (A) 0 ng, (B) 10 ng, (C) 100 ng, and (D) 1000 ng of mAb per milliliter and then scored for rosette formation with opsonized sRBC's.

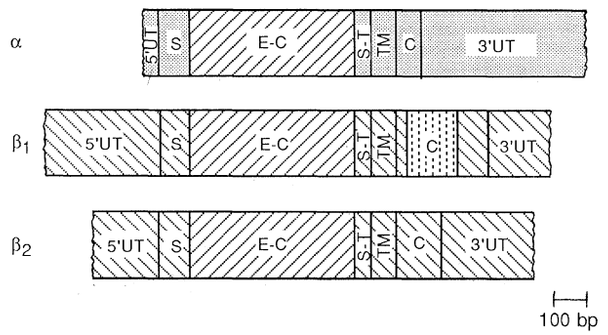

Fig. 11. Summary of the structure of the FcγR cDNA's of macrophage and T cells. Two genes have been identified, designated α and β, which demonstrate 95 percent identity in the predicted extracellular domain (E-C), indicated by the diagonal shading. The β_1 and β_2 sequences are identical with the exception of a 138-nucleotide insertion, indicated by the hatching, in the cytoplasmic domain (C) of the β_1 sequence, giving rise to an additional 46 amino acids in this domain. UT, untranslated sequences; S, the signal sequence; S-T, a serine-threonine rich region preceding the transmembrane (TM) domain; and C, the cytoplasmic domain.

antibody to the effector cells. In this article, we address the structural and functional heterogeneity of FcγR's, both in terms of isotype specificity and function of lymphocyte and macrophage FcR's. Several studies (*12–14*) suggest that the lymphocyte FcγR that is reactive with mAb 2.4G2 has specificity for murine IgG1, IgG2b, and IgG2a, whereas the comparable macrophage receptor does not bind IgG2a (*6, 10*). In addition, it appears that the functions of FcγR's on macrophages and lymphocytes probably differ. Our studies may provide a structural basis for the heterogeneity of FcγR's on the different cell types, and may account for the differences in isotype specificity. In addition, the structural heterogeneity described in our study may account for the difference in function in terms of the two receptors transducing different signals in response to the same extracellular ligands.

We have identified two genes that encode Fcγ receptors. One of these, referred to as α, is expressed in macrophage cell lines as well as normal peritoneal macrophages. The second gene, referred to as β, is expressed in both macrophage and lymphocyte (T and B) lines. These genes encode transmembrane proteins with an internally repeated domain each containing two glycosylation sites, and a potential intrachain disulfide loop of 42 to 45 amino acids. This predicted structure is consistent with in vivo labeling studies, which have suggested the presence of four N-linked glycosylation sites. While the extracellular domains of the α and β genes are 95 percent homologous, the transmembrane and cytoplasmic domains encoded by the two genes are totally different, an indication that the different functions of lymphocyte and macrophage FcγR's may derive in part from different signaling mechanisms.

There is, however, still another level of complexity in this gene system, since there appear to be cell-specific splicing mechanisms that may result in altered protein products. Although the β gene is transcribed in both lymphocyte and macrophage cell lines, detailed analysis of a T cell transcript (β_1) revealed an additional 138 nucleotides, which result in an insertion of 46 amino acids in the cytoplasmic domain of FcγRβ_1. Ribonuclease protection experiments have shown that the β_1 transcript is seen in both T cell and B cell lines, and not in the P388D1 or RAW 264.7 macrophage cell lines and most probably arises from an alternative splicing pathway for the β gene. The early macrophage lines P388 and WEHI 3A have β transcripts of the same apparent mobility as the lymphocyte cell lines, and this similarity suggests that β_1 splicing may be developmentally regulated during macrophage maturation. The consequences of the insertion are not understood, but it is possible that the longer cytoplasmic domain in the β_1 FcγR interacts differently with cytoplasmic or membrane proteins involved in signal transduction. The structures of the three transcripts derived from the α and β genes are summarized in Fig. 11.

The sequences obtained for these receptors demonstrate that the FcγR belongs to the immunoglobulin supergene family, as has been shown for the poly-IgA receptor, which functions in the transport of IgA across epithelial cells (*32*). Characteristic invariant residues of the V_κ framework regions (FR1, 2, and 3) are conserved in the FcγR sequences. Apart from the overall immunoglobulin homology, several other significant homologies were identified for the FcγR. The extracellular domains of the α and β genes are most homologous to the MHC class II protein E_β in its β_2 domain. The other significant homology detected for the transmembrane domain of the α subunit, but not the β chain, is with one of the transmembrane domains of the α subunit of the acetylcholine receptor, consistent with the observations of Young *et al.* (*33*) and Nelson *et al.* (*34*) who have reported ion channel activity in response to binding of ligand to FcγR's.

The observation that the FcγR is homologous to class II histocompatibility antigens may account for the role of this molecule in the Mls-driven T cell proliferative response as mimicry of the MHC-mediated proliferative response. Hibbs *et al.* (*16*) and Mark *et al.* (*15*) have shown that Ly-17, an antigen closely associated with the Mls locus on mouse chromosome 1, is identical to the FcγR immunoprecipitated by mAb 2.4G2. The chromosomal location of the α and β genes to chromosome 1 (*35*) is consistent with these results. However, the four reported alleles of this locus among different inbred mouse strains do not correlate with the restriction fragment length polymorphism observed for the α gene (Fig. 7A). This may reflect the need for additional polymorphic markers to segregate these alleles or the possibility that more than one determinant is involved in the allelic response to the Mls.

Finally, the isolation and characterization of immunoglobulin binding factors (IBF) that are reported to suppress primary antibody plaque forming responses by B cells is relevant to our studies. These IBF's, which have been isolated from T cells and T cell lines, are reported to bear the same antigenic determinants recognized by mAb 2.4G2 as do membrane FcγR's on T cell lines (*36*). The mechanism for secretion or release of IBF is not known, nor is the precise structure of the molecule or molecules involved.

The identification of the structure of these FcR's presents the opportunity to explore the contribution of the various domains of this molecule to its function on macrophages and lymphocytes. We have demonstrated that transfection of the sequences encoded by these cDNA's results in the appearance of surface proteins capable of binding immunoglobulin complexes. The subclass specificity of binding for the α and β chains and consequences of ligand binding for the transfectants remain to be determined.

Note added in proof: Since completion of these studies we have learned that Lewis *et al.* (*51*) have isolated and sequenced a cDNA clone for a Fcγ2b/γ1R from a mouse macrophage line and have found a sequence identical to that called β_2 in our study.

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"I've got the perfect diet to avoid fats and carcinogens: reptile tails, powdered insects, fungi, and worms."