Molecular Cloning of a Novel Murine Cell-surface Glycoprotein Homologous to Killer Cell Inhibitory Receptors*

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We have isolated a cDNA clone encoding a novel murine cell-surface glycoprotein. This polypeptide is predicted to be composed of a signal peptide of 23 amino acids, an extracellular region of 620 amino acids that contains six immunoglobulin-like domains with five potential N-glycosylation sites, a transmembrane sequence of 20 amino acids, and a cytoplasmic tail of 178 amino acids with four sets of sequences similar to the immunoreceptor tyrosine-based inhibition motif. The relative molecular mass of the mature polypeptide is calculated to be 90,520 Da. The polypeptide, designated as p91, shows striking homologies to human killer cell inhibitory receptors, a murine gp49B1 protein, a bovine Fcγ2 receptor, and a human Fcε receptor. The mRNA of p91 was especially abundant in murine macrophages. Western blot analysis using p91-specific anti-peptide sera detected a 130-kDa polypeptide in macrophages. Surface biotinylation and immunoprecipitation analysis verified the surface expression of the translation products on COS-1 cells transfected with the p91 cDNA, but the cells failed to show any Fc binding activity.

Recent findings have shown that human killer cell inhibitory receptors (KIRs), including p58 (1), natural killer (NK)-associated transcripts (2), and NK1B1 (3, 4), are novel members of the immunoglobulin superfamily, which is a group of proteins with widely varying distribution and functions (for review, see Refs. 5–9). KIRs are expressed exclusively on NK cells and T cells and play a pivotal role in the recognition of polymorphic major histocompatibility complex class I molecules on target cells and the delivery of the inhibition signal to the cell interior by means of a mechanism involving phosphorylation of a specific tyrosine residue in their immunoreceptor tyrosine-based inhibition motif (ITIM) and recruitment of src homology 2-containing phosphatases such as SHP-1 (10). KIRs show significant sequence homologies to a human Fc receptor for IgA (FcaR) (11) as well as a mouse gp49 protein (12, 13). Recently, Katz et al. (14) performed a functional analysis of the gp49B1 cDNA (15), the murine counterpart of human FcεRI, and suggested that the involvement of the molecule could be inhibitory to mast cell activation when it was co-ligated with the high affinity receptor for IgE (FcεRI). Thus, these recent observations have shed light on the field of research on structural, functional, and evolutionary relationships among each member of the KIR family or its relatives. It is also possible to speculate that some other unidentified molecules of the KIR group remain to be characterized, especially in a murine system.

During the course of experiments to obtain a hypothetical murine counterpart of human FcεRI, we have cloned complementary DNAs coding for a novel molecule that exhibits exceedingly high sequence homologies to human KIRs (1–4), the murine gp49B1 protein (15), bovine Fcγ2R (16), and human FcεRI (11). The possible function of this gene product, designated as p91 due to the molecular mass of its mature polypeptide backbone, has been discussed.

EXPERIMENTAL PROCEDURES

Screening and Isolation of cDNA Clones—An 885-base pair XbaI fragment from a cDNA for human FcεRI (a generous gift from Drs. D. L. Sylvestre and J. V. Ravetch, Sloan-Kettering Institute, New York) (11) was labeled with a random primer labeling kit (Takara Shuzo Co., Otsu, Japan) and [α-32P]dCTP (specific activity, ~3000 Ci/mmoll; Amersham Corp.) and was used to screen a genomic library constructed from 4–5-week-old female 129/Sv mouse liver DNA in the Lambda FixII vector (Stratagene). 5 × 105 plaques were screened under less stringent conditions (hybridization in 35% formamide at 42 °C and washing in 2 × SSC at room temperature) (17). Plaque purification was completed for six positive clones. A 0.5-kilobase pair (kb) BamHI fragment from one of the genomic clones containing an exon encoding the first and third Ig-like extracellular (EC) domains, respectively, were used to isolate cDNA clones from an oligo(dT)-primed cDNA library in the Uni-Zap XR vector prepared from B10.A mouse thiglycolate-elicited peritoneal macrophages (Stratagene) under stringent conditions.

DNA Sequencing—The cDNA clones were subcloned into the plasmid pUC19 or pBluescript (Stratagene) and sequenced in both strands by the dideoxy chain termination method (18) using a Cy5 AutoRead sequencing kit and an ALFExpress DNA sequencer (both from Pharmacia Biotech Inc.).

Construction of Expression Vector for p91—A 2.7-kb cDNA insert of clone p91-55 (see below) containing a whole coding sequence of p91 was excised with XbaI and KpnI and subcloned into the XbaI-KpnI site of pUC19. The resulting plasmid was digested partially with EcoRI; the 2.6-kb fragment was gel-purified; and the fragment was then recloned into the EcoRI site of the expression vector pCExV-3 (19, 20). The orientation of the cDNA insert was confirmed by nucleotide sequencing. The expression vectors for FcγRIIB, FcγRIII, and FcεRIγ were gifts from Dr. T. Kurosaki (Kansai Medical College, Osaka, Japan).

RNA Blot Analysis—Total RNA from various mouse tissues (21) was...
The probes were labeled by random priming with ^32PdCTP and used for hybridization under stringent conditions. The blots were washed and exposed to an imaging screen and analyzed with a BAS1000 Bio-Imaging Analyzer (Fuji Photo Film Co., Tokyo, Japan).

Preparation of Antisera—For preparation of antisera to p91, the synthetic peptides acetyl-SLPKPIILQVDPDSVSC-amide (peptide 1) and acetyl-CSSGAEFPTRQGELQK-amide (peptide 2), corresponding to amino acids 2–17 of the extracellular domain and amino acids 661–676 of the cytoplasmic domain of p91, respectively, were synthesized; the carboxyl- or amino-terminal cysteine was added to facilitate coupling to carrier protein. Each of the peptides was coupled to keyhole limpet hemocyanin; the conjugate was suspended in saline, emulsified by mixing with an equal volume of Freund’s adjuvant, and injected into three to four subcutaneous dorsal sites of 3–9-month-old New Zealand White rabbits. Booster immunizations were administered two to three times at 3-week intervals, and 1 week after the final booster immunization, blood was collected. Peptide synthesis and immunizations were performed by Quality Control Biochemicals, Inc. (Hopkinson, MA).

Cell Culture—Bone marrow-derived mast cells were prepared as described previously (22). Briefly, bone marrow cells from femurs and tibias of 6–8-week-old C57BL/6 or B10.A mice were cultured for 3–4 weeks in RPMI 1640 medium plus 10% FCS with 75 μg/ml heparin in a 10-cm plastic dish. The cell suspension was layered on 1 g/ml recombinant interleukin-3 (rIL-3; Immunex Corp.) and cultured in Dulbecco’s modified Eagle’s medium (Sigma), and peritoneal exudate cells were harvested 4 days later. The cells were suspended in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum. Thyroglobulin-elicted peritoneal macrophages were isolated as described previously (22). Briefly, mice were injected intraperitoneally with 1.5 ml of 5% thioglycolate (22). Various cells from mouse tissues including splenocytes, macrophages were subjected to the experiments. NK cells were induced by a modified DEAE-dextran method combining with an osmotic shock treatment as described previously (25). Alternatively, intact SRBCs were incubated with mouse anti-SRBC whole serum to opsonize total Ig. These antibodies for opsonization were used at non-agglutinating titers. A 50-μl aliquot of the opsonized SRBCs at a 1% suspension was added to a 1-ml culture of macrophages or transfected COS-1 cell (–1 × 10^5 cells) monolayers and incubated for 60 min at 4°C for rosetting. The cells were washed extensively with PBS (–) for elimination of non-rosetted SRBCs and fixed in 0.1% paraformaldehyde (PFA) for 15 min at 4°C. The percentage of rosetting SRBCs was calculated by counting positive cells in at least three microscopic fields selected randomly. >120 cells in total were counted for each determination.

Results

Isolation of Genomic and cDNA Clones—We initially attempted to clone a hypothetical murine counterpart of human FceRI (11). We screened a murine genomic DNA library with a human FceRI cDNA fragment as a probe under low stringent, cross-hybridization conditions and isolated six clones. Restriction mapping and sequencing analyses of these clones revealed that they were derived from a single genomic locus, but sequence similarity to human FceRI was not high enough to enable us to easily identify other exon sequences. Therefore, we next tried to isolate cDNA clones of the transcript from this genomic locus. A preliminary RNA blot analysis using samples from various mouse tissues suggested that the macrophages could be a rich source of the corresponding mRNA species. A mouse cDNA library made from thyroglobulin-elicted macrophages was screened with a 0.5-kb BamHI fragment and a 0.4-kb HindIII fragment from one of the mouse genomic clones as hybridization probes. From 1 × 10^6 phage plaques, 56 signals positive for both probes were obtained, seven of which were randomly selected and subjected to plaque purification. Restriction mapping analysis indicated that these seven clones could be divided into three groups; we characterized three clones (cDNA-55, cDNA-76, and cDNA-17) representative of each group in more detail (Fig. 1). The group containing cDNA-55 contained four clones including cDNA-55 (harboring a 2.7-kb cDNA insert), whereas the second group, containing cDNA-76 (a 2.5-kb insert), was composed of two members, and cDNA-17 (a 2.9-kb insert) was the sole constituent of its group.

Analysis of Predicted Amino Acid Sequence—Fig. 1A shows the nucleotide and deduced amino acid sequences of cDNA-55. The nucleotide sequence is composed of an open reading frame of 2523 nucleotides, 120 nucleotides of 3′-untranslated sequence containing a classical AATAAA polyadenylation signal, and a poly(dA) tail. Efforts to isolate cDNA clones containing further upstream sequence compared with cDNA-55 were not successful. We have assigned the first ATG (nucleotides 1–3) as the translation initiation codon. The sequence of the 23 amino acids starting with the initiating methionine exhibits hydrophobic features characteristic of the signal peptide (Fig. 1, A and B). The first amino acid of the mature polypeptide was assigned as Gly-1 (Fig. 1A) according to the general rule for signal peptide cleavage sites (26), although we cannot exclude the possibility that Ser-2 is the mature N-terminal residue. The deduced polypeptide is composed of 841 amino acids including a signal peptide of 23 amino acids. Nucleotides 1930–1989 encode a highly hydrophobic sequence composed of 20 amino acids

2 Available at http://www.genome.ad.jp/.
FIG. 1. Nucleotide sequence of mouse p91 cDNA. A, nucleotides were determined from the nucleotide sequence of cDNA-55. The sequence is numbered on the right from the A residue in the translation start codon. The deduced amino acid sequence is shown below the nucleotide sequence in one-letter code and is numbered on the left, with position 1 set to the predicted start of the mature protein; negative numbers are assigned to the signal peptide. The polyadenylation signal AATAAA is shaded. Potential sites for N-glycosylation are boxed. Cysteine residues in the mature extracellular region are circled. A hydrophobic stretch of 20 amino acids predicted to span the plasma membrane is underlined. The four core sequences YX2(L/V) in the ITIM are double-underlined.

B, shown is the Kyte-Doolittle hydrophobicity/hydrophilicity profile (46) of the
Cloning of p91, a Murine Homolog of Human KIRs

predicted p91 protein. Numbers on the horizontal axis are amino acid positions. C, shown is a schematic diagram of the predicted protein domains of p91 and its subtypes. The protein structure is subdivided into the signal peptide (S) in black boxes, extracellular Ig-like domains (EC1–EC6), the transmembrane domain (TM) in black boxes, and the cytoplasmic domain (CP) in shaded boxes. The positions of 12 cysteines forming potential disulfide bonds (C–C) in the extracellular domain are shown. Stars indicate the positions of the ITIM-like core sequences. The nucleotide sequence of cDNA-17 contained the sequence 5'-GAATCTGTTATTAGAC-3' after nucleotide 946, thus yielding a stop codon (underlined in the above sequence). cdNA-55 has a 9-base insertion (5'-GTGAGGAGG-3') between nucleotides 2023 and 2024, possibly due to a cloning artifact because other clones characterized had no insertion at this position. The nucleotide sequence of cdNA-76 has a deletion from nucleotides 1553 to 1855, possibly due to splicing out of an exon.
stream of the G residue at position 946, thus yielding a putative polypeptide with 296 amino acids containing only the first three EC domains (Fig. 1C).

Detection of p91 mRNA in Mouse Tissues—A blot containing 5 μg of total cellular RNA from various mouse tissues and cultured cells was probed with the 1491-base pair EcoRI-EcoRI fragment from cDNA-55, which corresponds to nearly the whole EC region of p91 (Fig. 3). A predominant 3.0-kb mRNA species was detected in bone marrow-derived mast cells, macrophages, splenocytes, mesenteric lymph node cells, embryonic fibroblast cells, and peritoneal resident cells, with less intense hybridization to an RNA species of 2.0-kb. Interestingly, NK cells contained only the 2.0-kb mRNA species. The longer RNA species presumably represents mature mRNA for the full-length p91 polypeptide and possibly includes mRNA subtypes of cDNA-76 and cDNA-17. It is not known whether the smaller RNA species encodes other truncated forms of p91 or whether it originates from a cognate, but different gene.

Detection of p91 Polypeptide in Cultured Cells and Macrophages by Western Blotting and Immunoprecipitation—To confirm the expression of p91 protein in cells, we analyzed various tissues from B10.A mice including macrophages as well as COS-1 cells transfected with p91 cDNA using anti-p91 antibodies. As shown in Fig. 4A, Western analyses of lysates from COS-1 cells transfected with p91 detected two major bands with apparent molecular masses of 130 and 110 kDa and faint multiple bands with apparent molecular masses of 200–300 kDa. These bands were not detected by preimmune rabbit serum (Fig. 4A, panel a) or by anti-p91 antiserum in the presence of antigenic peptide 2, corresponding to part of the cytoplasmic tail (residues 661–676) (panel c). In contrast, incubation of anti-p91 antiserum with EC domain peptide 1 did not affect the intensity of the bands (Fig. 4A, panel d). The 130-kDa band was also detected in lysates from thioglycolate-elicited peritoneal macrophages and peritoneal resident cells (Fig. 4A, panel b). The specificity of binding was again verified by the disappearance of the band in the presence of peptide 2 (Fig. 4A, panels a and c). Essentially the same results were obtained when the antiserum against p91-specific peptide 1 (corresponding to residues 2–17) was used (data not shown).

Extracts from COS-1 cells transfected with p91 cDNA were immunoprecipitated by antiserum raised against p91-specific peptide 2 and then detected by Western blotting using the same antiserum. As shown in Fig. 4B, two major bands with apparent molecular masses of 130 and 110 kDa were detected. These signals almost completely disappeared when competing peptide 2 was added to the incubation mixture with primary antiserum at the immunoprecipitation step, thus verifying the specificity of the binding reaction. It is possible that the 130-kDa species represents a highly glycosylated form of the mature p91 molecule, whereas the smaller species may represent a less glycosylated one. The nature of the 200–300-kDa
polypeptide species detected in the transfected COS-1 cells is not known.

Fig. 4B shows an immunoprecipitation analysis of COS-1 transfec tant using anti-peptide 2 IgG-coated Sepharose beads and Western detection with the same antiserum. Again, the two major bands of 130 and 110 kDa were observed in an anti-p91 antiserum precipitate and a precleared total lysate when detected by anti-p91 antiserum.

To verify the cell-surface expression of p91 translation products, we first labeled cell-surface proteins with biotin, followed by immunoprecipitation and detection by Western blotting (Fig. 4C). In COS-1 cells transfected with p91 cDNA, anti-p91 peptide 2 serum detected a 110-kDa signal as well as a very faint 130-kDa band. Thus, the p91 translation product is shown to be expressed on the cell surface.

Functional Analysis of p91—p91 shows significant homologies to human KIRs (1–4), murine gp49B1 (15), human FcγRs (11), and bovine Fcγ2R (16). Based on the apparent homologies to FcRs, we first postulated that p91 may bind an Fc portion of Ig. To address this issue, we examined the Ig binding activities using COS-1 cells transfected with the p91 cDNA (Table II). We could not detect, however, any Ig binding activity of such COS-1 cells transfected with p91 cDNA.

A second possibility is that p91 may bind unidentified molecules of the Ig superfamily such as major histocompatibility complex class I molecules on target cells like human KIRs and suppress the cellular activity of macrophages. The deduced protein structure shows striking similarities to human KIRs (1–4), murine gp49B1 (15), human FcγR (11), and bovine Fcγ2R (16). Based on the apparent homologies to FcRs, we first postulated that p91 may bind an Fc portion of Ig. To address this issue, we examined the Ig binding activities using COS-1 cells transfected with the p91 cDNA (Table II). We could not detect, however, any Ig binding activity of such COS-1 cells transfected with p91 cDNA.

DISCUSSION

We have isolated a cDNA clone that encodes a novel murine cell-surface glycoprotein, p91, preferentially expressed on macrophages. The deduced protein structure shows striking similarities to human KIRs (1–4), murine gp49B1 (15), human FcγR (11), and bovine Fcγ2R (16). Based on the apparent homologies to FcRs, we first postulated that p91 may bind an Fc portion of Ig. To address this issue, we examined the Ig binding activities using COS-1 cells transfected with the p91 cDNA (Table II). We could not detect, however, any Ig binding activity of such COS-1 cells transfected with p91 cDNA.

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in various immune functions such as antibody production by B cells (41), mast cell degranulation (41), and T cell proliferation (42, 43). Thus, ITIMs may mediate inhibitory effects by recruiting tyrosine phosphatases or an inositol phosphatase that could reverse and/or suppress tyrosine and inositol phosphorylation at one or more steps of signal transduction cascades that activate cellular functions. Furthermore, a recent finding by Katz et al. (14) suggests that the mouse gp49B1 molecule may have an inhibitory role in mast cell activation, although the counterligand of gp49B1 is uncertain. They have proposed that gp49B1 is a mast cell inhibitory receptor that belongs to murine counterparts of human KIRs.

The p91 molecule does contain the four repeats of ITIM-like structure (Fig. 1, A and C), one of which matches the ITIM consensus sequence under stringent criteria. Therefore, it should be clarified whether these Tyr residues in p91 ITIM-like sequences are controlled by phosphorylation/dephosphorylation upon stimulation of the p91 molecule in vivo. It also should be tested whether p91 on macrophages could suppress cellular functions such as phagocytosis and release of proinflammatory mediators.

We have also cloned cDNAs different from cDNA-55, one of which, cDNA-76, possibly encodes a protein with similar membrane topology to p91 except that this molecule has five Ig-like extracellular domains due to splicing out of the exon encoding the sixth EC domain of p91. Another clone, cDNA-17, possibly encodes a soluble secretory protein with three Ig-like domains. This type of molecular structure is reminiscent of the mouse IgE-binding factor, a soluble form of low affinity FcE for IgE (FcεRII or CD23). FcεRII possesses a C-type lectin domain with IgE binding capacity and part of a mouse endogenous retroviral sequence (44). It is interesting to speculate that a soluble form of the p91 molecule may have some physiological roles like the IgE-binding factor, which has been suggested to control IgE production and inflammation (45). The significance of these two subtypes of p91 molecules remains to be clarified.

Recent analysis of molecular structures and functions of KIRs has opened an intriguing field of research on functional and evolutionary correlation and ligand binding specificities of these molecules or related molecules such as gp49B1. Elucidation of the counterligand and physiological function of the macrophage p91 molecule as well as that of a possible cognate gp49B1 molecule will facilitate the understanding of the functional rationale for the specialization of such molecules of the KIR group on widely varying cells.

REFERENCES

1. Wagtmann, N., Biasconi, R., Cantonii, C., Verdiann, S., Malnati, M. S., Vitale, M., Bottino, C., Moretta, L., Moretta, A., and Long, E. O. (1985) Immunity 2, 439–449
2. Colonna, M., and Samardis, J. (1995) Science 268, 405–408
3. Litvin, V., Gumperz, J., Parham, P., Phillips, J. H., and Lanier, L. L. (1994) J. Exp. Med. 180, 537–543
4. Phillips, J. H., Gumperz, J. E., Parham, P., and Lanier, L. L. (1995) Science 268, 403–405
5. Rouet, D. H., and Hold, W. (1995) Cell 82, 697–700
6. Leibson, P. J. (1995) Immunity 3, 5–8
7. Yokoyama, W. M. (1993) Curr. Opin. Immunol. 5, 67–73
8. Lanier, L. L., and Phillips, J. H. (1996) Immunol. Today 17, 86–91
9. Long, E. O., Colonna, M., and Lanier, L. L. (1996) Immunol. Today 17, 100
10. Burnhult, D. N., Scharenberg, A. M., Wagtman, N., Rajagopalan, S., Berrada, K., Yi, T., Kinet, J.-P., and Long, E. O. (1996) Immunity 4, 77–85
11. Maliszewski, C. R., March, C. J., Schoenborn, M. A., Gimpel, S., and Shen, L. (1995) J. Exp. Med. 172, 1665–1672
12. Katz, H. R., Benson, A. C., and Austen, K. F. (1989) J. Immunol. 142, 919–926
13. Arm, J. P., Gurish, M. F., Reynolds, D. S., Scott, H. C., Gartner, C. S., Austen, K. F., and Katz, H. R. (1993) J. Biol. Chem. 268, 15966–15973
14. Castells, M. C., Wu, X., Arm, J. P., Austen K. F., and Katz, H. R. (1994) J. Biol. Chem. 269, 8393–8401
15. Zhang, G., Young, J. R., Tregaskes, C. A., Sopp, P., and Howard, C. J. (1995) J. Immunol. 155, 1534–1541
16. Sambrook, J., Frisch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
17. Sanger, F., Nicklen, S., and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 5463–5467
18. Okayama, H., and Berg, P. (1983) Mol. Cell. Biol. 3, 280–289
19. Miller, J., Marek, T. R., Leonard, W. J., Greene, W. C., Shervah, E. M., and Germain, R. N. (1985) J. Immunol. 134, 4212–4217
20. Takai, T., Li, M., Sylvester, D., Clynes, R., and Ravetch, J. V. (1994) Cell 76, 519–529
21. Jeynor, A. L. (1993) in Gene Targeting (Joyner, A. L., ed) pp. 36–41, Oxford University Press, New York
22. Groettrup, M., Ungewiss, K., Azogui, O., Palacios, R., and Owen, M. J. (1994) Cell 75, 283–294
23. Takai, T., and Ohmori, H. (1990) Methods Mol. Cell. Biol. 2, 82–90
24. von Heijne, G. (1984) J. Mol. Biol. 173, 243–251
25. Bairoch, A., and Boeckmann, B. (1994) Nucleic Acids Res. 22, 3578–3580
26. Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. (1990) J. Mol. Biol. 215, 403–410
27. Ravetch, J. V., Luster, A. D., Weisnakh, R., Kochan, J., Pavlove, A., Portnoy, D. A., Hulmes, J., Pan, Y.-C. E., and Unkeless, J. C. (1986) Science 234, 718–725
28. Ishioka, N., Takahashi, N., and Putnam, F. W. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 2363–2367
29. Gennarini, G., Cibelli, G., Rougon, G., Mattei, M.-G., and Goridis, C. (1989) J. Cell Biol. 109, 775–788
30. Hessson, C., Moy, P., Tizard, R., Chisholm, P., Williams, C., Wyck, M., Burke, L., Miyake, K., Kincaide, P., and Lobb, R. (1992) Biochem. Biophys. Res. Commun. 183, 163–169
31. Dondi, G. M., Justement, L. B., Delibrias, C. C., Matthews, R. J., Lin, J., Thomas, M. L., and Fearon, D. T. (1995) Science 269, 242–244
32. Marengere, L. E. M., Waterhouse, P., Duncan, G. S., Mittrucker, H. W., Feng, G. S., and Mak, T. W. (1996) Science 272, 1170–1173
33. Thomas, M. L. (1995) J. Exp. Med. 181, 1953–1956
34. Olcese, L., Lang, P., Vely, F., Cambiaggi, A., Marquet, D., Bley, M., Hippen, K. L., Biasconi, R., Moretta, A., Moretta, L., Cambier, J. C., and Vivier, E. (1996) J. Immunol. 156, 4539–4543
35. Muta, T., Kurosaki, T., Misulovin, Z., Sanchez, M., Nussenzeew, M. C., and Ravetch, J. V. (1994) Nature 368, 70–73
36. Daeron, M., Latour, S., Malbec, O., Espinosa, E., Fina, P., Pasmans, S., and Fridman, W. H. (1995) Immunity 3, 635–646
37. D'Ambrosio, D., Hippen, K. L., Minkoff, S. A., Mellman, I., Pan, G., Siminovitch, K. A., and Cambier, J. C. (1995) Science 269, 293–297
38. Ono, M., Bolland, S., Tempest, P., and Ravetch, J. V. (1996) Nature 383,
41. Takai, T., Ono, M., Hikida, M., Ohmori H., and Ravetch, J. V. (1996) *Nature* **379**, 346–349
42. Waterhouse, P., Penninger, J. M., Timms, E., Wakeham, A., Shahinian, A., Lee, K. P., Thompson, L. B., Griesser, H., and Mak, T. W. (1995) *Science* **270**, 985–988
43. Tivol, E. A., Borriello, F., Schweitzer, A. N., Lynch, W. P., Bluestone, J. A., and Sharpe, A. H. (1995) *Immunol. Rev.* **3**, 541–547
44. Toh, H., Ono, M., and Miyata, T. (1985) *Nature* **318**, 388–389
45. Ishizaka, K. (1984) *Annu. Rev. Immunol.* **2**, 159–182
46. Kyte, J., and Doolittle, R. F. (1982) *J. Mol. Biol.* **157**, 105–132
47. Dayhoff, M. O., Schwartz, R. M., and Orcutt, B. C. (1978) in *Atlas of Protein Sequence and Structure* (Dayhoff, M. O., ed) Vol. 5, pp. 345–352, National Biomedical Research Foundation, Silver Spring, MD