Identification of Residues within the Extracellular Domain 1 of Bovine Fcγ2R Essential for Binding Bovine IgG2

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H. Craig Morton§§, Chris J. Howard¶¶, Anne K. Storset¶, and Per Brandtzaeg‡

From the §Laboratory of Immunohistochemistry and Immunopathology, Institute of Pathology, University of Oslo, Rikshospitalet, N-0027 Oslo, Norway, ¶Division of Immunology and Pathology, Institute for Animal Health, Compton, Newbury, Berkshire RG20 7NN, United Kingdom, and ‡Department of Pharmacology, Microbiology and Food Hygiene, Norwegian School of Veterinary Science, N-0033 Oslo, Norway

Neutrophils and monocytes in cattle express a novel class of immunoglobulin Fc receptor, specific for bovine IgG2 (bIgG2), termed bFcγ2R. In cows, the ability of neutrophils to kill immunoglobulin-opsonized microorganisms appears to depend largely on this subclass, whose interaction with bFcγ2R initiates the killing process. bFcγ2R is a transmembrane glycoprotein consisting of two extracellular immunoglobulin-like domains, followed by a 19-amino acid membrane-spanning region and a short cytoplasmic tail. Although related to other mammalian FcRs, bFcγ2R belongs to a novel gene family that includes the human killer cell inhibitory receptor and FcεRI (CD89) proteins. We have shown previously (Morton, H. C., van Zandbergen, G., van Kooten, C., Howard, C. J., van de Winkel, J. G., and Brandtzaeg, P. (1999) J. Exp. Med. 189, 1715–1722) that these proteins (and unlike other FcγRs), bFcγ2R binds bIgG2 via the membrane-distal extracellular domain 1 (EC1). In this present study, we introduced mutations into the predicted loop regions of the EC1 domain and assayed the resulting bFcγ2R mutants for their ability to bind bIgG2. Our results indicated that the bFcγ2R binding site lies within the predicted F-G loop region of the EC1 domain. Furthermore, single amino acid mutational analysis of this region identified Phe-82 and Trp-87 as being critical for bIgG2 binding.

Receptors for the Fc regions (FcRs) of IgS expressed on circulating phagocytes provide a crucial link between humoral and cellular immunity. Cross-linking of FcRs by Ig-containing immune complexes triggers a wide variety of effector mechanisms such as phagocytosis, antibody-dependent cellular cytotoxicity, and release of cytokines and other mediators of inflammation (1, 2). Cattle possess fourCY genes (one of which is a pseudogene) giving rise to the expression of three subclasses of bIgG (bIgG1, bIgG2, and bIgG3) (3–8). Several bIgG-binding proteins have been isolated, of which three are considered homologues to FcγRI (CD64), FcγRII (CD32), and FcγRIII (CD16) expressed by many other mammalians including humans (7, 9–11). A homologue of the MHC class I-like FcR, FcRN, which is responsible for maternal transfer of IgG in humans, mice, and rats, is also found in cattle (12). In addition, there is a novel type of FcγR specific for bIgG2, termed bFcγ2R (13). This FcR is genetically more closely related to a novel family of human proteins that includes FcεRI (CD89) and the KIR, monocyte/macrophage Ig-like receptor, and Ig-like transcripts/LIR families of receptors, which all probably arose by a series of duplication events from a single ancestral gene (14).

To date little is known about the respective roles of the bIgG subclasses and the different cattle FcγRs in the triggering of immune effector functions. In terms of humoral immunity, it has been reported that bIgG1 is a better activator of complement (at least in vitro), which may be linked to the observation that most breeds of cattle have a higher serum concentration of bIgG1 than bIgG2 (3, 15). Importantly, however, when cell-mediated immune reactions were examined in cattle, bIgG2 seemed to perform at least as well as, and sometimes even better than, bIgG1. For example, in an in vitro assay system that measured the ability of bovine macrophages and neutrophils to kill Ig-opsonized Mycoplasma bovis, both isotypes performed equally when macrophages were employed as effector cells. However, when neutrophils were used, only bIgG2 was able to provide effective killing (16). These results could be explained in part by the observation that bovine monocytes and macrophages express FcγRs for both bIgG isotypes, whereas neutrophils express high numbers of receptors for bIgG2 but few or none for bIgG1 (17). Thus, although it is still difficult to assess the biological role of the bovine homologues of FcγRI, FcγRII, and FcγRIII, due partly to the lack of specific monoclonal antibodies (mAbs), it is clear that bIgG2 and its receptor on neutrophils and macrophages, bFcγ2R, is an important triggering molecule for cellular effector functions in cattle.

bFcγ2R consists of two extracellular Ig-like domains, followed by a transmembrane region and a short cytoplasmic tail. Uniquely, for an FcγR, bFcγ2R has been shown to bind bIgG2 via its membrane-distal EC1 domain (18). All other FcγRs so-far characterized bind IgG via the membrane proximal EC2 domain (19, 20). However, it now appears that ligand binding via the EC1 domain may be a typical characteristic of many other members of the gene family to which bFcγ2R belongs, including CD89, KIR, and LIR-1 (18, 21, 22).

In this study we have used site-directed mutagenesis to identify regions of the EC1 domain of bFcγ2R involved in the binding of bIgG2. Our results showed that the bIgG2 binding site lies within the putative F–G loop of the EC1 domain. Moreover, Phe-82 and Trp-87 were identified as critical for...
Identification of the IgG2 Binding Site of bFcy2R

Expression and Ligand Binding Characteristics of bFcy2R—Bovine neutrophils have previously been shown to express a specific FcR for bFcy2R. This receptor has been identified as bFcy2R, and a murine mAb of the IgM isotype (CC-G24) has been raised.2 CC-G24 bound specifically to bFcy2R expressed at high levels on bovine neutrophils (Fig. 1, panel A). CC-G24 was also able to specifically block binding of HA-bIgG2 to bovine neutrophils (Fig. 1, panel B). HA-bIgG2 was shown to almost completely inhibit binding of bIgG2-coated beads to purified bovine neutrophils; this suggested that bFcy2R was the only relevant receptor for bFcy2G2 expressed by these cells (Fig. 1, panel C). CC-G24 also efficiently recognized bFcy2R expressed transiently on the surface of COS-1 cells (Fig. 1, panel D). Using Fugene 6 we routinely obtained transfection efficiencies in the range of 15 to 40% (as measured by either CC-G24 or HA-bIgG2 binding), which was considered sufficient for our FACS-based bFcy2G2 binding assay. For rosetting analysis, however, we decided to enrich for bFcy2R-expressing cells. Naturally we could not use mAb CC-G24 to identify bFcy2R-expressing COS-1 cells, because this mAb binds to the EC1 domain and blocks bFcy2G2 binding (see panels B and F, and see Ref. 18). Therefore, to select for cells that had taken up DNA, COS-1 cells were co-transfected with the bFcy2R plasmids and a vector encoding GFP (Fig. 1, panel E). Our initial experiments using this co-transfection protocol demonstrated that the COS-1 cell population expressing the highest level of GFP (and therefore being the most fluorescent) contained the highest percentage of bFcy2R-positive cells (data not shown). Therefore, a gate was defined (see Fig. 1, panel E) that allowed the purification of the COS-1 cells with the highest fluorescence intensity. For the wild type bFcy2R, we found that 70–90% of the (GFP+ cells purified according to the above protocol also expressed bFcy2R, as assessed by their ability to bind bIgG2-coated beads (Fig. 1, panel F). Furthermore, prior incubation of bFcy2R / GFP+ COS-1 cells with mAb CC-G24 almost completely abolished binding of these cells to bIgG2-coated beads (Fig. 1, panel F).

Design, Construction, and Expression of bFcy2R Mutants—Bovine Fcy2R is a member of the Ig gene superfamily of proteins and is closely related to several human members of this family, including the KIRs, Ig-like transcripts, and the human FeR (CD89) (14). Several three-dimensional structures of KIR proteins have now been described, thus identifying the secondary structure composition of the Ig domains. Therefore, because of the highly conserved nature of the Ig-like domain structure, an amino acid alignment of the Ig domains of KIR and bFcy2R was generated (Fig. 2). When the relative positions of the KIR β-strands were superimposed on this alignment the conservation of residues in comparable regions of bFcy2R was apparent. Because clustering of conserved residues in areas of structural significance may indicate a common Ig domain structure, these regions also likely form β-stranded secondary structures in bFcy2R (and probably other family members). In addition, it is now recognized that amino acids that form ligand binding sites within Ig-like domains are predominately located within the loop regions. The β-strands, on the other hand, tend to be more involved in the preservation of the overall Ig-like domain structure. It is probably for this reason that amino acids in the loop regions of Ig-like domains tend to be less conserved than those in the strands (see Fig. 2). Thus, we initially selected a series of amino acids predicted to lie in and around several putative loop regions of bFcy2R as targets for mutagenesis. We then introduced three and four amino acid mutations into these regions to reveal which of them might be

2 C. J. Howard, unpublished data.
involved in bIgG2 binding and thus might be candidates for further studies. Regions identified during this first round of screening were further analyzed by the introduction of single base pair mutations. We first introduced a series of three or four amino acid mutations into the B–C, C–C/H11032, E–F, and F–G loops, because these were the largest loops and thus the areas of greatest divergence (see Fig. 2). The C–C/H11032 and F–G loops were especially interesting when we considered what was already known concerning the location of ligand binding sites within other members of the gene family to which bFcγ2R belongs. The C–C/H11032 loop has been shown to be important for MHC class I recognition by the p58 KIR, and the F–G loop is reported to contain the IgA binding site of CD89 (21, 23). A further mutant was generated by deleting part of the (elongated) bFcγ2R EC1–EC2 linker region (the E–R del mutant), to study its contribution (if any) to bIgG2 binding. This mutant was constructed, because recent structural data have shown that the angle of the hinge between EC1 the EC2 domains of the KIR proteins can vary (possibly influencing ligand binding and function), and may partly depend on the composition and length of this linker region.

The bIgG2 Binding Site of bFcγ2R Lies in the F–G Loop—Mutant bFcγ2R cDNAs transfected to COS-1 cells were analyzed by FACS for surface expression, as measured by binding of the bFcγ2R-specific mAb CC-G24. At the same time, the expressed receptors were analyzed for their ability to bind small HA-bIgG2 complexes in solution, also by flow cytometry (see "Materials and Methods"). Analysis of the panel of EC1 domain mutants showed that only mutations in and around the F–G loop region of this domain influenced bIgG2 binding (Fig. 3). Mutation of amino acids SHF at positions 80–82 and VWN at positions 85–87 abolished binding of HA-bIgG2. Mutations in the B–C loop (NTK; positions 35–37), the C–C/H11032 loop (KEGD; positions 43–46), and the E–F loop (NVRE; positions 68–71) did not affect HA-bIgG2 binding. It is interesting to note, however, that whereas the NVRE mutant could bind HA-bIgG2, it was apparently not recognized by the bFcγ2R-specific mAb CC-G24. The number of cells transfected with the KEGD mutant detected by mAb CC-G24 was also reduced (compared with those positive with HA-bIgG2). Together, these observations suggested that the epitope recognized by mAb CC-G24 lies close to, or within, the E–F loop. In addition, our data also suggest that the epitope for this mAb may also include, to a lesser extent, part of the C–C’ loop.

Our first round of mutagenesis experiments thus identified several stretches of amino acids important for bIgG2 binding.
Mutation of amino acids SHF (position 80–82) and VNW (position 83–85) to alanine both produced mutant bFcγ2R molecules that were expressed at the cell surface but unable to bind bIgG2 (Fig. 3A). Our data therefore suggested that residues within the F–G loop region were necessary for binding of bIgG2. We next selected four additional amino acids within these regions for further individual mutational analysis. Two of these amino acids are predicted to lie at the beginning of the F–G loop (His-81 and Phe-82) whereas the other two lie further along this loop (Asn-86 and Trp-87) (see Fig. 2). Asn-86 was also interesting from a structural point of view, because it lies within a possible N-glycosylation addition sequence (Asp-X-Ser/Thr or N-X-(S/T) with the one letter code; see Fig. 2). It is well known that changes in the glycosylation patterns of proteins can adversely affect their expression and function. Results, however, demonstrated that the N86A mutant was expressed at the cell surface and readily able to bind HA-bIgG2. Similarly, the H81A mutant was also expressed at the cell surface and able to bind HA-bIgG2. Conversely, we found that even though the F82A and W87A mutants were indeed expressed at the cell surface (albeit on a low number of cells) and readily able to bind bIgG2-coated beads, they proved entirely unable to bind HA-bIgG2 (Fig. 3B).

The consequences of deleting the linker region between the EC1 and EC2 domains of bFcγ2R (the E–R del mutant) were more difficult to interpret. Although our FACS-based assays showed that this mutant was not recognized by the mAb CC-47797, we did detect a small percentage of cells able to bind HA-bIgG2. By generating and expressing a panel of bFcγ2R proteins carrying specific mutations within their membrane-distal EC1 domains, we have identified residues essential for forming the bIgG2 binding site. Several amino acids located immediately prior to the predicted start of the F–G, loop as well as residues within the loop itself, were shown to be critical for bIgG2 binding. In particular we found that mutation of either Phe-82 or Trp-87 to alanine completely abolished binding to bIgG2.

Introduction of point mutations into the loop regions of FeRs and Igs has provided a great deal of information regarding their structure and function (19). These proteins are composed of several globular domains linked by extremely variable stretches of amino acids that make up the flexible loop regions. Thus, mutagenesis of residues within these loop regions probably does not adversely disturb native protein structure. Importantly, the majority of bFcγ2R mutants described here were still able to efficiently bind bIgG2 and were still recognized by an anti-bFcγ2R mAb. Even when the mAb recognition site was disrupted (in the NVRE and KEGD mutants) these receptors were still able to bind bIgG2. Furthermore, the mutants that were unable to bind bIgG2 were still recognized by the mAb. Therefore, we feel that mutagenesis of residues within the EC1 domain of bFcγ2R are unlikely to have adversely affected overall protein structure, allowing assessment of the relative contribution of the mutated residues to the bIgG2 binding site.

We also chose to delete the stretch of amino acids that forms...
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Fig. 3. Binding of CC-G24 and HA-bIgG2 to \(bFc\gamma2R\) mutants. COS-1 cells were transfected with mutated \(bFc\gamma2R\) cDNAs as indicated. Two days thereafter the cells were harvested and stained with either CC-G24 or HA-bIgG2. Mock-transfected COS-1 cells were used as negative control. Density plots of forward scatter (FSC) against FL1 (FITC) are shown. The percentage of positive cells is indicated in the top left of each panel. A, mutants generated during the first round of mutagenesis. The loop in which the mutation is located is indicated. The positions of the mutated amino acids are shown in parenthesis. B, F–G loop mutants generated during the second round of mutagenesis. The results shown are representative of at least three separate experiments.

The linker region between the EC1 and EC2 domains of \(bFc\gamma2R\). The linker region of \(bFc\gamma2R\) is longer than in other related receptors and may have a structural significance. Thus deletion of this region may have more severe consequences for the overall structural integrity of this receptor. Our results showed that whereas surface expression of the E–R del mutant could not be detected by the anti-\(bFc\gamma2R\) mAb, a small proportion of transfectedants were still able to bind \(bIgG\). One explanation for these observations could simply be that the E–R del mutant was only expressed by a few cells (because of low transfection efficiency) but when expressed was readily able to bind \(bIgG\). However, an alternative explanation may be that deletion of the EC1–EC2 linker region results in an aberrantly folded protein unable to be expressed with the correct conformation at the cell surface. The few cells detected in our assay may result from surface expression of incorrectly assembled \(bFc\gamma2R\) molecules where the \(bIgG\) binding site is still exposed.

Bovine \(Fc\gamma2R\) is closely related to a family of human genes found on chromosome 19 within the leuokocyte receptor complex (LRC) that includes the KIR, monocyte/macrophage Ig-like receptor, Ig-like transcripts/LIR, and CD89 proteins (24, 25). In support of this close genetic relationship, the \(bFc\gamma2R\) gene has been mapped to bovine chromosome 18, which is reported to correspond to human chromosome 19 (26). The genes of the LRC are only slightly less closely related genetically to the other mammalian FcRs than they are to each other. Moreover, available crystal data suggest that proteins encoded in the LRC are structurally quite similar to FcRs (FcγRII, III, and FcεRI) (14). However, despite this high degree of homology, the location of the ligand binding sites within these molecules varies considerably. The binding site for human IgG within FcγRII and FcγRIII lies predominantly in the EC2 domain, close to the EC1/EC2 hinge region (27–29). A similar EC2 site within FcεRI is important for binding IgE (30, 31). In both these cases, residues within the F–G loop of the EC2 domain are involved (19). In contrast, the ligand binding sites within the LRC-encoded proteins studied to date have been shown to lie predominantly in the membrane-distal EC1 domains (18, 21–23). However, the location of the ligand binding sites varies even among LRC-encoded proteins. The specificity of the p58 KIR protein for its MHC I ligand has been shown to depend on a single residue located in the C–C' loop of EC1 (marked with an open triangle in Fig. 2) (21). More recent data have suggested that additional residues located around the EC1/EC2 hinge region of the KIRs are involved in maintaining high affinity binding to their MHC I ligands. According to the solved crystal structure of the KIR proteins, these residues all lie on the top face of the molecule (22). On the other hand, ligand binding sites within CD89 and LIR-2 (the only other LRC-encoded proteins yet characterized) are located in a different region of EC1. For both these proteins residues within and around the putative F–G loop are critical for high affinity binding to ligand, whereas exposed residues in other parts of EC1 can also contribute (22, 23, 32).

Although mutation of residues in and around the F–G loop of the \(bFc\gamma2R\) EC1 domain adversely affected \(bIgG2\) binding, mutations in other putative loops had little effect. Mutation of residues in the predicted B–C loop (NTK mutant), the C–C' loop (KEGD mutant), and the E–F loop (NVRE mutant) did not affect \(bIgG2\) binding. Thus mutation of residues around the KIR binding site in the C–C' loop had no effect on function. We did, however, notice that mutations introduced into the E–F loop abolished binding of the anti-\(bFc\gamma2R\) mAb CC-G24, whereas mutation of the C–C' loop produced receptors that bound the mAb poorly. These results suggest that the epitope for mAb CC-G24 includes (at least partly) the E–F loop region and may also involve (part of) the C–C' loop. It is interesting to note that if the three-dimensional structure of \(bFc\gamma2R\) proves to closely resemble that of the KIRs, these two loops would be
predicted to lie close together on the top of the EC1 domain (33).

We also compared the EC1 F–G loop sequence of bFcγ2R with the EC2 F–G loop sequence from other mammalian FcγRs for comparable IgG binding motifs. We noted that all low affinity FcγRs possess a conserved glycine (Gly-156) residue at the beginning of the EC2 F–G loop that is always preceded by a hydrophobic residue (Ile, Phe, Lys, or Val). Both these residues have been implicated in the binding of IgG (19). A similar motif, Ile-83–Gly-84, is present at a comparable position in the EC1 F–G loop of bFcγ2R. Thus we wondered whether these residues could also play a role in binding to bIgG2. However, our results obtained with the IGV mutant showed that these residues do not contribute to bIgG2 binding.

We also examined the bFcγ2R EC1 F–G loop sequence for similarities with the same region of CD89. Elegant mutagenesis studies with soluble CD89 molecules have shown that Arg-82 and His-85 are essential for IgA binding, whereas Arg-87 makes a minor contribution (23). Because of the nature of the amino acids involved in binding, it would appear that the CD89-IgA interaction is charge-based. Strikingly, our data demonstrate that amino acids at positions 82 and 87 of bFcγ2R are also essential for ligand binding, although the hydrophobic nature of the residues involved in the bFcγ2R-bIgG2 interaction suggests a different method of binding. Interestingly, as mentioned above, hydrophobic residues are thought to be important for ligand binding by other mammalian FcγRs, perhaps by the formation of a hydrophobic pocket to which the IgG is subsequently bound (19).

Therefore, it appears that the IgA binding site of CD89 and the bIgG2 binding site of bFcγ2R lie in almost identical areas of the molecules. One may wonder whether this could be explained by the nature of their ligands. It is known that bFcγ2R binds neither IgG1 nor human IgA (15). Conversely, bIgG2 binds poorly, or not at all, to other bovine FcγRs (10). The reason for this may be that bFcγ2R carries a 6-amino acid deletion within the lower hinge region at a site considered to form an FcR binding motif within all IgG molecules in which it is present, including bIgG1 (19, 34). Thus, bIgG2 has clearly evolved an FcR binding site distinct from other mammalian IgG molecules. Therefore, it is extremely interesting to note that the CD89 binding site within IgA is not located in the lower hinge region, as it is with IgG, but rather is located at the Co2/Co3 interface (35, 36). Unfortunately, it is not known where the bFcγ2R binding site of bIgG2 lies, although our results strongly suggest that it may perhaps be located at a similar position to that found in human IgA, i.e. the Cγ2/Cγ3 boundary.

Altogether, it is likely that the evolution of Ig binding sites within the EC1 domains of bFcγ2R and CD89 are specific adaptations that allow efficient binding to Ig molecules at sites distinct from the lower hinge region. At least for IgA, the Co2/Co3 region must be easily accessible to the EC1 domain within all the molecular forms of IgA (monomeric, dimeric, and secretory IgA) and not be obstructed by a J chain or a secretory component, because CD89 is able to bind all monomeric and polymeric forms (37). A possible reason for the relocation of the FcR binding site in these Ig molecules is that the lower hinge region may be blocked by other parts of the molecule. Supporting this theory is the recent observation that IgA1 may in fact adopt a more “T”-like shape, in contrast to the more “Y” shape more readily associated with IgG molecules, suggesting that the hinge region of IgA1 is (at least partly) obscured by the Fab arms hindering binding to this region (38). Moreover, molecular modeling of bovine IgG2 predicts that because of the deletion in the hinge region, bIgG2 is a highly compact molecule with a close positioning of the Fab and Fc domains; this would allow only very little or no angular or rotational movement of the Fab arms in relation to the Fc domain (34). Interestingly, IgA2, the second subclass of human IgA (which readily binds to CD89) also possesses a very short hinge region, and the structure and flexibility of this isotype probably more closely resembles that of bIgG2 (39).

In conclusion, we have identified residues in and around the F–G loop of the EC1 domain of bFcγ2R that are important for ligand binding. Specifically, we have shown that Phe-82 and Trp-87 are critical for the binding of bIgG2. The bIgG2 binding site of bFcγ2R is similar to the IgA binding site of the closely related human IgA Fc receptor, CD89. It is likely that these binding sites have evolved to ensure efficient ligand binding and effector cell activation by these FcRs.

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REFERENCES
1. Ravetch, J. V., and Kinet, J. P. (1991) Annu. Rev. Immunol. 9, 457–492
2. Daeron, M. (1997) Annu. Rev. Immunol. 15, 203–234
3. Butler, J. E. (1983) Vet. Immunol. Immunopathol. 4, 43–152
4. Butler, J. E., Heyermann, H., Frey, L. V., and Kiernan, J. (1987) Immunol. Lett. 16, 51–38
5. Knight, K. L., Suter, M., and Becker, R. S. (1988) J. Immunol. 140, 3654–3659
6. Symons, D. B., Clarkson, C. A., and Beale, D. (1989) Mol. Immunol. 26, 841–850
7. Bianchi, A., Butler, J. E., Hoofar, J., Howard, C., and Lind, P. (1996) Vet. Immunol. Immunopathol. 54, 25–31
8. Butler, J. E. (1998) Rev. Sci. Tech. 17, 43–70
9. Symons, D. B., and Clarkson, C. A. (1992) Mol. Immunol. 29, 1407–1413
10. Zhang, G., Young, J. R., Tregaskes, C. R., and Howard, C. J. (1994) Immuno- genetics 39, 425–427
11. Collins, R. A., Gelder, K. I., and Howard, C. J. (1997) Immunogenetics 45,
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12. Kacskovics, I., Wu, Z., Simister, N. E., Frenyo, L. V., and Hammarstrom, L. (2000) *J. Immunol.* 164, 1889–1897
13. Zhang, G., Young, J. R., Tregaskes, C. A., Sopp, P., and Howard, C. J. (1995) *J. Immunol.* 155, 1534–1541
14. Dennis, G. J., Kuhagawa, H., and Cooper, M. D. (2000) *Proc. Natl. Acad. Sci. U. S. A.* 97, 13245–13250
15. Howard, C. J., and Brownlie, J. (1979) *Res. Vet. Sci.* 27, 388–389
16. Howard, C. J. (1984) *Vet. Immunol. Immunopathol.* 6, 321–326
17. Morton, H. C., van Zandbergen, G., van Kooten, C., Howard, C. J., van de Winkel, J. G., and Brandtzaeg, P. (1999) *J. Exp. Med.* 189, 1715–1722
18. Wines, B. D., Hulett, M. D., Jamieson, G. P., Trist, H. H., Lay, C. S., and Hogarth, P. M. (2001) *J. Immunol.* 166, 1781–1789
19. Winter, C. C., and Long, E. O. (1997) *J. Immunol.* 158, 4026–4028
20. Carayannopoulos, L., Hexham, J. M., and Capra, J. D. (1999) *J. Mol. Biol.* 286, 1421–1447
21. Pleass, R. J., Dunlop, J. I., Anderson, C. M., and Wool, J. M. (1999) *J. Biol. Chem.* 274, 23508–23514
22. Mazengera, R. L., and Kerr, M. A. (1990) *Biochem. J.* 272, 159–165
23. Carayannopoulos, L., Hexham, J. M., and Capra, J. D. (1996) *J. Exp. Med.* 183, 1579–1586
24. Pleass, R. J., Dunlop, J. I., Anderson, C. M., and Wool, J. M. (1999) *J. Biol. Chem.* 274, 23508–23514
25. Winter, C. C., and Long, E. O. (1997) *J. Mol. Biol.* 266, 1421–1447
26. Khungland, H., Vage, D. I., and Lien, S. (1997) *Mamm. Genome* 8, 300–301
27. Maxwell, K. F., Powell, M. S., Hulet, M. D., Barton, P. A., McKenzie, I. F., Garrett, T. P., and Hogarth, P. M. (1999) *Nat. Struct. Biol.* 6, 437–442
28. Sondermann, P., Huber, R., and Jacob, U. (1999) *EMBO J.* 18, 1095–1103
29. Zhang, Y., Boesen, C. C., Radaev, S., Brooks, A. G., Fridman, W. H., Sauttes-Fridman, C., and Sun, P. D. (2000) *Immunity* 13, 387–395
30. Garman, S. C., Kinét, J. P., and Jardetzky, T. S. (1999) *Annu. Rev. Immunol.* 17, 973–976
31. Garman, S. C., Wurzburg, B. A., Tarchevskaya, S. S., Kinét, J. P., and Jardetzky, T. S. (2000) *Nature* 406, 259–266
32. Wines, B. D., Sardjono, C. T., Trist, H. H., Lay, C. S., and Hogarth, P. M. (2001) *J. Immunol.* 166, 1781–1789
33. Winter, C. C., and Long, E. O. (1997) *J. Immunol.* 158, 4026–4028
34. Chapman, T. L., Heikema, A. P., West, A. P., and Bjorkman, P. J. (2000) *Immunity* 13, 727–736
35. Carayannopoulos, L., Hexham, J. M., and Capra, J. D. (1996) *J. Exp. Med.* 183, 1579–1586
36. Pleass, R. J., Dunlop, J. I., Anderson, C. M., and Wool, J. M. (1999) *J. Biol. Chem.* 274, 23508–23514
37. Mazengera, R. L., and Kerr, M. A. (1990) *Biochem. J.* 272, 159–165
38. Bohm, M. K., Wool, J. M., Kerr, M. A., and Perkins, S. J. (1999) *J. Mol. Biol.* 266, 1421–1447
39. Kerr, M. A. (1990) *Biochem. J.* 271, 285–296