Fcα/μ receptor mediates endocytosis of IgM-coated microbes

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Abstract

IgM is the first antibody to be produced in a humoral immune response and plays an important role in the primary stage of immunity. Here, we describe a mouse Fc receptor, designated Fcα/μ receptor, and its human homologue which bind both IgM and IgA with intermediate or high affinity. The Fcα/μ receptor is constitutively expressed on the majority of B-lymphocytes and macrophages. Cross-linking of Fcα/μ receptors expressed on a pro-B cell line Ba/F3 transfectant with soluble IgM or IgM-coated microparticles induced internalization of the receptor. Moreover, the Fcα/μ receptor mediated endocytosis of *Staphylococcus aureus* coated with IgM by primary B-lymphocytes. Thus, the Fcα/μ receptor is involved in the primary stage of the immune response against microbes.

The interaction of antibody-antigen complexes with cells results in a wide array of immune responses, including antibody-dependent cellular cytotoxicity, mast cell degranulation, phagocytosis, cell proliferation, antibody secretion and enhancement of antigen presentation[1-3]**.** These interactions are initiated through binding of the Fc portions of antibodies or immune complexes to cell surface receptors on hematopoietic cells. For example, IgG antibodies bind the Fcγ receptor on macrophages and neutrophils mediating phagocytosis of opsonized microorganisms and/or secretion of cytokines involved in inflammation. IgE antibodies induce the release of chemical mediators from mast cells and basophils by binding to the Fcε receptor. Considerable progress has been made in elucidating the structural and functional diversity of the Fc receptors for IgG and IgE. Results obtained in Fc receptor-deficient mice show an important role for those receptors on host immunity, allergy or autoimmunity[4-10]. Analysis of mice lacking the gene for the α subunit of the high-affinity IgE receptor, Fcε receptor I, reveals a defect in IgE-triggered passive anaphylaxis due to the absence of the Fcε receptor I on mast cells and basophils[4]. Similarly, mice with a disrupted γ gene from the IgE Fcε receptor I or the IgG Fcγ receptor I and III exhibit identical phenotypes[5]. Moreover, Fc receptor γ gene knockout mice lack IgG-mediated phagocytosis by macrophages[5] and the ability to mount the Arthus reaction[6]. In contrast to these triggering Fc receptors, the Fcγ receptor IIb inhibits BCR-triggered lymphocyte proliferation, antibody secretion and lymphokine secretion[1]. Antibody and anaphylactic responses to antigen are elevated in mice lacking the Fcγ receptor IIb gene[7].

Because IgM is the first antibody to be produced in a humoral immune response, it may play an important role in the innate stage of immunity. In addition, natural antibodies, involved in the prevention of pathogen dissemination to vital organs, are mainly IgM[11]. However, it is not known how IgM protects against infection. The existence of functional Fc receptors for IgM on subpopulations of human and rodent T, B and NK cells has been suggested[12-17]. However, by contrast to γ- and ε-Fc receptors, the structural and functional characteristics of the Fcμ receptor are unclear because a Fcμ receptor gene has not been cloned, although a polymeric Ig receptor able to bind IgM and IgA has been described[18]. Similarly, although a human Fcα receptor (CD89) gene has been characterized[19], a homologous rodent Fcα receptor has not yet been identified. In the present investigation, we have identified a murine Fc receptor, designated Fcα/μ receptor, and its human homologue that binds both IgA and IgM. We describe the functional and molecular characteristics of this receptor.

Results

Characteristics of the mouse and human Fcα/μ receptors

To identify a Fc receptor for IgM, we screened COS-7 cells transfected with a cDNA library prepared from the mouse T cell leukemia, BW5147, by using a mouse IgM monoclonal antibody (mAb) as a probe. We isolated a cDNA clone of 2,361 bp that contains an open reading frame encoding a type I transmembrane protein with a 32 amino acids (aa) leader sequence, a 423 aa extracellular domain, a 20 aa transmembrane domain and a 60 aa cytoplasmic region (**Fig. 1a**). The extracellular domain has four potential sites for NH_2-linked glycosylation, suggesting that the receptor is a glycoprotein. A pair of cysteine residues in the extracellular domain is flanked by the consensus sequence for Ig-like domains (**Fig. 1a, b**), indicating that this molecule, designated mFcα/μ receptor, is a member of the Ig supergene family. A human cDNA with homology to the mFcα/μ receptor was isolated from a human lymph node cDNA library and was found to encode a protein with 49% amino-acid identity to the mFcα/μ receptor. These genes were mapped by fluorescence in-situ hybridyzation (FISH)[20] to syntenic regions of mouse chromosome 1 (1F) and human chromosome 1 (1q32.3), near several other Fc receptors, including Fcγ receptors I to III, the Fcε receptor and the polymeric Ig receptor[21]. No tyrosines are present in the cytoplasmic domain, but there is a di-leucine motif in the mFcα/μ receptor that may be involved in receptor internalization. The Fcα/μ receptor is unique, showing no significant homology to other proteins or nucleotide sequences in the GenBank, EMBL, or DDBJ databases. However, we observed a motif in the Ig-like domain that is conserved in the first Ig-like

domain of human, bovine and rodent polymeric Ig receptors which binds to the Fc of IgA and IgM[22-24] (**Fig. 1b**). Overall, the Fcα/μ receptor has less than 10% amino acid homology with the mouse and human polymeric Ig receptor.

COS-7 cells were transfected with the Flag-tagged mouse Fcα/μ (mFcα/μ) receptor cDNA at the NH_2-terminus, which resulted in surface expression of the receptor as detected by immunofluorescence using a mAb to Flag (anti-Flag) (data not shown). Immunoprecipitation of the Flag-tagged mFcα/μ receptor on the COS-7 transfectants using anti-Flag revealed an ~70 kD protein in reducing and non-reducing conditions (**Fig. 1c**).

Expression of the Fcα/μ receptor

To analyze the expression of the mFcα/μ receptor in various tissues we used reverse transcription-polymerase chain reaction (RT-PCR). The mFcα/μ receptor is expressed in several tissues, including thymus, spleen, liver, kidney, small and large intestines, testis, and placenta (**Fig. 2a**). The mFcα/μ receptor transcripts were also detected in the T cell leukemia cell lines, RLmale1 and BW5147 (data not shown). To further analyze the expression of the mFcα/μ receptor on hematopoietic cells, RNA was obtained from lymphohematopoietic progenitor and lineage-committed cells in the bone marrow, thymus and spleen. The mFcα/μ receptor transcripts were expressed in $B220^+$ B cells in bone marrow and spleen, but not in lymphohematopoietic progenitors, granulocytes, T cells or NK cells (**Fig. 2b**). To confirm the cell surface expression of the mFcα/μ receptor protein, we generated a mAb (rat IgG isotype) to the mFcα/μ

receptor. Analysis of spleen cells by flow cytometry showed that the Fcα/μ receptor is expressed on the majority of B cells and macrophages but not on granulocytes, T cells or NK cells (**Fig. 2c**).

The Fcα/μ receptor binds IgM and IgA

To determine the binding specificity of this receptor, we established a Ba/F3 transfectant stably expressing the mFcα/μ receptor. Flow cytometric analysis showed that the transfectant specifically bound several mouse IgM mAbs and both dimeric and monomeric IgA mAbs but not any IgG mAbs tested including mouse IgG1, IgG2a, IgG2b and IgG3 (**Fig. 3a**). The mFcα/μ receptor transfectant also bound rat IgM and IgA mAbs, and human IgM (data not shown). By contrast, the parental Ba/F3 cells, which do not express the Fcα/μ receptor, did not bind any of these mAbs (**Fig. 3a**). Moreover, the Ba/F3 transfectant bound the Fc, but not F(ab')$_2$, fragment of human IgM (**Fig. 3c**). Therefore, the Fcα/μ receptor is a specific Fc receptor for the mouse IgM and IgA and cross-reacts with human and rat IgM, and rat IgA. To confirm the binding specificity, the Ba/F3 transfectant was pre-incubated with a pure mouse IgM or rat IgA mAbs and then stained with a different FITC-conjugated mouse or rat IgM mAbs. Binding of FITC-labeled mouse IgM and IgA mAbs was inhibited by pre-incubation of the transfectant with IgM or IgA mAbs (**Fig. 3b**), indicating that the molecule is a specific receptor for IgM and IgA. Binding of IgM and IgA to the mFcα/μ receptors expressed on the Ba/F3 transfectant was also determined with ^{125}I-labeled ligands. The mFcα/μ receptor bound with high

affinity any species of IgM tested. In addition, the mFcα/μ receptor also bound mouse IgA with intermediate affinity (**Fig. 3d**).

The Fcα/μ receptor and endocytosis of IgM-coated beads

The cytoplasmic domain of the mFcα/μ receptor contains a di-leucine motif (**Fig. 1a**), implicated in endosome and lysosome targeting of diverse proteins and involved in agonist-induced internalization[25-28]. A di-leucine motif is involved in Fcγ receptor IIB-mediated endocytosis[3, 29]. To investigate whether the Fcα/μ receptor internalizes upon cross-linking, the Ba/F3 transfectants expressing the Fcα/μ receptor were incubated with FITC-labeled mouse IgM and then cross-linked with goat anti-mouse IgM at 4^{o}C or 37 ^{o}C for 1 h. Some of the transfectants were treated with trypsin to remove the FITC-labeled mouse IgM bound to the cell surface Fcα/μ receptors that did not undergo internalization. Treatment with trypsin significantly decreased the fluorescence intensity of the transfectant expressing the Fcα/μ receptor which was incubated at 4^{o}C after cross-linking (**Fig. 4a**). By contrast, the fluorescence remained constant in the transfectant which was incubated at 37^{o}C after cross-linking, despite treatment with trypsin. This suggests that cross-linking the Fcα/μ receptor with IgM induced internalization of the Fcα/μ receptor during culture at 37^{o}C.

We next examined whether the Fcα/μ receptor is involved in endocytosis. Ba/F3 transfectants expressing the mFcα/μ receptor were incubated with IgM-coated fluorescent beads at 37^{o}C. After extensive washing and treatment with

trypsin, we analyzed the cells by flow cytometry. A subpopulation of the Ba/F3 transfectants containing fluorescent beads was detected (**Fig. 4b**). The fluorescent beads were also seen in the cytoplasm of the transfectant using immunofluorescent microscopy and confocal scanning laser microscopy (**Fig. 4c-e**), indicating that the Fcα/μ receptor is involved in endocytosis of IgM-coated beads. Transfectants incubated with fluorescent beads not coated with IgM were not engulfed by the transfectants (data not shown).

The di-leucine motif of the Fcα/μ receptor

We examined whether the di-leucine motif at residues 519 and 520 in the cytoplasmic portion of the mFcα/μ receptor is involved in the internalization of the Fcα/μ receptor. We made Ba/F3 transfectants stably expressing Fcα/μ receptors mutated at residue 519, 520 or both and incubated them with IgM-coated green fluorescent beads at 37^{o}C. The fluorescent beads were not detected in any of the Ba/F3 transfectants expressing mutant Fcα/μ receptors (**Fig. 4b**). These results indicate that both leucines at residue 519 and 520 are required for internalization by the mFcα/μ receptor. Several other Fc receptors, including FcγRI, FcγRIIIA, FcεRI, and FcαRI, also mediate endocytosis[30-33] that is dependent on the associated subunit Fc receptor γ chain[34]. We did not observe an association of the Fcα/μ receptor with the γ chain. Because the human Fcα/μ receptor does not have a di-leucine motif in its cytoplasmic domain; other elements in the human receptor probably serve this function.

The Fcα/μ receptor mediates endocytosis by primary B-lymphocytes

To examine the biological significance of the Fcα/μ receptor-mediated endocytosis, mouse spleen cells were incubated with immune complexes composed of FITC-labeled *S. aureus* bacteria coated with IgM or IgG antibodies to*S. aureus*. After overnight culture, the cells were treated with trypsin, stained with PE-conjugated anti-B220 mAb and analyzed by flow cytometry. While $B220^+$ spleen cells cultured with the IgG *S. aureus* immune complex did not show any specific green fluorescence, a subset of $B220^+$ cells cultured with the*S. Aureus* IgM immune complex showed detectable levels of fluorescence. When spleen cells were precoated with rat anti-mFcα/μ receptor mAb and incubated with the IgM-*S. aureus* complex, $B220^+$ cells did not show any specific fluorescence (**Fig. 4f**). The $B220^+$ B cells incubated with the IgM-*S. aureus* complexes were isolated by flow cytometry and examined by fluorescence microscopy. These cells contained one or more FITC-labeled *S. aureus* bacteria in the cytoplasm (**Fig. 4g,h**). These results indicate that the mFcα/μ receptor mediates endocytosis of IgM-coated microbial pathogens.

Discussion

Prior reports suggested the existence of functional Fc receptors for IgM on murine and human T, B and NK cells[12-17]. However, functional and molecular characteristics of these Fcμ receptors have remained elusive. Among them, a human Fcμ receptor, which is expressed on activated B cells, but not on macrophages, is a ~60 kD glycosyl phosphatidylinositol (GPI) -linked protein[15]. The Fcα/μ receptor we describe here is ~70 kD and a transmembrane type of glycoprotein expressed on both B cells and macrophages. These data suggest that these Fc receptors are different. The localization of the Fcα/μ receptor gene on chromosome 1 suggested that the Fcα/μ receptor may be evolutionary related to Fcγ receptors, the Fcε receptor and the polymeric Ig receptor, but distinct from the human Fcα receptor (CD89) whose gene is mapped to chromosome 19[21].

We have shown that the Fcα/μ receptor is expressed not only on hematopoietic cells but also on variable non-hematopoietic organs, including liver, kidney, small and large intestines, testis and placenta. Although the polymeric Ig receptor, which also binds IgM and IgA, is expressed on epithelial cells, it is unclear whether the Fcα/μ receptor is expressed on epithelial cells or on infiltrated hematopoietic cells in these organs. Similarly, although purified thymic T lymphocytes are deficient in the expression of the Fcα/μ receptor, thymic tissue is positive for Fcα/μ receptor expression.

Nonetheless, we have demonstrated the functional expression of the Fcα/μ receptor on B lymphocytes. Transfection of Ba/F3 cells with the Fcα/μ receptor allowed these pro-B cells to engulf IgM-coated particles.

Moreover, primary B-lymphocytes uptake IgM-coated microbial pathogens, such as *S. aureus*, through the Fcα/μ receptor, showing the physiological relevance of this receptor. These results suggest at least two kind of the Fcα/μ receptor-mediated immune responses. The Fcα/μ receptor may be involved in the protection of bacterial infections by allowing phagocytosis of IgM-coated organisms and in facilitating of the subsequent antigen processing and presentation to helper T lymphocytes. Reports have demonstrated that mice deficient in the secretory form of IgM exhibit delayed development of specific IgG antibodies to T cell-dependent foreign antigens[35] and dissemination of micropathogens in peripheral organs but not in secondary lymphoid organs[11]. These reports suggest that uptake of IgM-antigen immune complexes by B-lymphocytes and macrophages through the Fcα/μ receptor may play an essential role in the priming of helper T lymphocytes. Analysis of mice lacking the Fcα/μ receptor gene will help to analyze the physiological role of this receptor.

Methods

Antibodies

For binding assays, anti-human CD18 mAb (L130, mouse IgG1), anti-human CD3 mAb (UCTH1, mouse IgG1), anti- human CD45 mAb (H130, mouse IgG1), anti-human CD71 mAb (M-A712, mouse IgG2a), anti-human CD11c mAb (LeuM5, mouse IgG2b), anti-human CD2 mAb (Leu5b, mouse IgG2b), anti-human CD52 mAb (DX8, mouse IgG3), anti-human CD57 mAb (Leu7, mouse IgM), DX18 mAb (mouse IgM), M18-254 mAb (mouse IgA), DX5 mAb (rat Ig M), TX2 mAb (rat IgM), TX1 mAb (rat IgA), human IgM (obtained from a patient with macroglobulinemia, a gift from Dr. Takachika Azuma (Science Univ. of Tokyo, Japan), and human IgM Fc fragment (Rockland) were used. Anti-human CD3, anti-human CD45, anti-human CD71 and M18-254 mAbs were purchased from PharMingen (San Diego, CA). DX5, DX8, DX18, TX1 and TX2 mAbs were generated in our laboratories. For flow cytometric analysis and sorting of the spleen, bone marrow and thymus, control IgG, anti-mouse TCR$\alpha\beta$, anti-mouse CD45R/B220, anti-mouse CD11b (Mac1) mAbs, anti-mouse Sca-1, anti-mouse c-kit, anti-Ly6G (Gr-1), TER119, anti-mouse CD90 (Thy-1), anti-NK1.1, anti-mouse CD4 and anti-mouse CD8 were used (PharMingen). An anti-mouse Fcα/μ receptor mAb (TX6) was generated by fusing the Sp2/0 myeloma cell line with splenocytes from a rat immunized with Ba/F3 transfectants expressing the mouse Fcα/μ[36].

Reverse transcription- polymerase chain reaction (RT-PCR)

RNA was subjected to reverse transcription and PCR using Fcα/μ receptor-specific primers (5'-AGTGTTACCACGAGTGAAGG-3' and 5'-TTCCATCATCAGGGTCTTGG-3') or *GAPDH*-specific primers (5'-TGCACCACCAACTGCTTAG-3' and 5'-GGATGC AGGGATGATGTTC-3'). Amounts of cDNA were measured by using an ABI7700 analytical thermal cycler (Perkin-Elmer Applied Biosystem, Foster City, CA). TaqMan Rodent GAPDH Control Reagent (Perkin-Elmer Applied Biosystem) was used to standardize results. Conditions used for PCR were: 40 cycles of 20 sec denaturation (94^{o}C), 20 sec annealing (55^{o}C), and 20 sec extension (72^{o}C).

Establishment of Ba/F3 transfectants

The Fcα/μ receptor cDNA was subcloned into the pMX-NEO retroviral vector (kindly provided from Toshio Kitamura, DNAX) using the Eco RI (5') and Not I (3') cloning sites. To generate site-directed mFcα/μ receptor mutants at residues L^{519} and/or L^{520}, PCR primers, which contained a codon for A^{519} (GCT) and /or A^{520} (GCT) instead of L^{519} (CTT) and L^{520} (CTT), respectively, were designed. The PCR products were subcloned into the pMX-NEO vector. All mutant cDNAs were verified by sequencing. Ba/F3 cells stably expressing wild type or mutant Fcα/μ receptors were established[37].

Scatchard Analysis

Antibodies were labeled by chloramine-T-catalyzed iodination and purified by gel-filtration chromatography on a PD-10 column (Sephadex G-25

M) (Pharmacia Biotech). Binding of Ba/F3 transfectants with ^{125}I -labeled antibodies and scatchard analysis were performed[17].

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Internalization Assay

Ba/F3 cells expressing the Fcα/μ receptor were stained with FITC-labeled mouse IgM (DX18 mAb), followed by cross-linking with goat anti-mouse IgM (Pharmingen) at 4^{o}C or 37 ^{o}C for 1 h. Some cells were treated with 0.1% trypsin and analyzed by flow cytometry. For internalization of IgM-coated beads, Ba/F3 cells expressing the wild type or mutant Fcα/μ receptor were incubated at 37^{o}C for overnight with yellow-green fluorescent beads (FluoSpheres Fluorescent Microspheres, 0.5 μm sized in diameter, Molecular Probes, Eugene, Oregon), which had been conjugated with mouse IgM (DX18 mAb) by as per manufacturers instructors. The cells were then washed with PBS and treated with 0.1 % trypsin to remove beads bound to the cell surface. The cells were centrifuged onto microscope slides, rapidly air-dried, and then analyzed using fluorescent and confocal scanning laser microscopes. To analyze endocytosis of the immune complexes, green fluorescent *S. aureus* (Molecular Probe) were incubated with either medium alone, anti-*S. aureus* mouse IgM or IgG mAbs (QED Bioscience Inc.). *The S. aureus* were then incubated overnight with spleen cells from C57Bl/6 mice pretreated with anti-mouse FcγRIII/II mAb (Pharmingen) to block interactions with Fcγ receptors. In some experiments, the anti-Fcα/μ receptor mAb (TX6) was also used to block the interaction with the Fcα/μ receptor. The spleen cells were then treated with 0.1% trypsin, stained with PE-conjugated anti-B220 mAb and analyzed by flow

cytometry. $B220^+$ B lymphocytes expressing green fluorescence were sorted, cytospinned onto slides and analyzed by using a fluorescent microscope.

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Figure Legends

Figure 1. Molecular and biochemical characterization of Fcα/μ receptors. (**a**) Predicted amino acid sequences of human and mouse Fcα/μ receptors. The putative leader and transmembrane domain are underlined. The predicted binding site for IgA and IgM is boxed. Potential NH_2-linked glycosylation sites in the extracellular domain are outlined. The arrowheads indicate potential cysteine residues involved in disulfide bonding of the Ig-like domains. A di-leucine motif in the cytoplasmic portion is shown in bold letters. The cDNA sequence data is available from EMBL/GenBank/DDBJ under accession number E15470 (human) and AB048834 (mouse). (**b**) Comparison of the amino acid sequences of the predicted binding sites for IgA and IgM of the Fcα/μ receptors and the polymeric Ig receptors. (**c**) The mFcα/μ receptor was immunoprecipitated with anti-Flag from COS-7 cells transfected with a cDNA encoding the Fcα/μ receptor tagged with Flag at the NH_2-terminus. Precipitates were analyzed under reducing and non-reducing conditions.

Figure 2. Expression of the mFcα/μ receptor. (**a, b**) Analysis of mFcα/μ receptor expression by RT-PCR. cDNA, adjusted to comparable quantities using a GAPDH control, were prepared from tissues or cells, as indicated. These cDNA or water (used as a negative control) were used as templates for RT-PCR. (**c**) Analysis of mFcα/μ receptor expression on mouse spleen and peripheral blood cells by flow cytometry. Spleen cells or peripheral blood cells from C57/BL6 mice were stained with biotin-conjugated anti-mouse Fcα/μ receptor (TX-6 mAb) and the FITC-conjugated mAbs indicated, followed by

allophycocyanin (APC)-conjugated streptavidin. An electronic gate, based on characteristic light scattering profiles, was set on mononuclear cells, lymphocytes and granulocytes. Greater than 99% of spleen and peripheral blood cells stained with control Igs were present in the lower left quadrant of the contour plots (data not shown). Data are representative of several independent experiments.

Figure 3. Binding of immunoglobulins to the mFcα/μ receptor. (**a**) Ba/F3 cells or a Ba/F3 transfectants expressing the mFcα/μ receptor were incubated with several FITC-labeled mouse immunoglobulins, as indicated, and analyzed by flow cytometry. (**B**) The Ba/F3 transfectants expressing the mFcα/μ receptor were pre-treated or not with an unconjugated mouse IgM (Leu7 mAb) or a rat IgA (TX1 mAb) and then stained with an FITC-labeled mouse IgM (DX18 mAb) or an FITC-labeled mouse IgA (M18-254 mAb). Controls are autofluorescence of the cells. Data are representative of several independent experiments. (**c**) The Ba/F3 transfectants expressing the mFcα/μ receptor were incubated with FITC-labeled intact human IgM and F(ab')2 and Fc fragment of human IgM, as indicated, and analyzed by flow cytometry. (**d**) Ligand binding assays. Ba/F3 transfectants expressing the mFcα/μ receptor were incubated with I^{125}-labeled mouse IgM (DX18 mAb), mouse IgA (M18-254 mAb), rat IgM (DX5 mAb) or human IgM for 2 h on ice, washed four times, and the cell-bound radioactivity was determined by using a gamma counter. Values for nonspecific binding, determined by binding to untransfected Ba/F3 cells, were subtracted. Data are plotted as Scatchard analyses and are representative

of several independent experiments.

Figure 4. Internalization of the mFcα/μ receptor. (**a**) The Ba/F3 transfectants expressing the mFcα/μ receptor were incubated with FITC-labeled mouse IgM (DX18 mAb) at 4^{o}C for 30 min, followed by cross-linking with goat anti-mouse IgM at 4^{o}C or 37^{o}C for 1 h. The cells were treated (+) or not (-) with 0.1% trypsin and analyzed by flow cytometry. The dotted line indicates the autofluorescence of the cells. (**b-e**) The Ba/F3 transfectants expressing wild type (WT) or site-directed mutant mFcα/μ receptors were incubated with FITC-conjugated mouse IgM (DX18 mAb) for 30 min and analyzed by flow cytometry (**b**, upper panel). The Ba/F3 transfectants were incubated overnight at 37^{o}C with mouse IgM (DX18 mAb)-coated green fluorescent beads, treated with 0.1% trypsin and analyzed by flow cytometry (**b**, lower panel), by fluorescent microscopy with light (**c**) or dark field illumination (**d**), or by confocal scanning laser microscopy (**e**). Whereas Ba/F3 transfectants bind comparable amounts of IgM to the cell surface (**b**, upper panel), the IgM-coated green fluorescent beads were only detected in the cytoplasm of transfectants expressing the wild type mFcα/μ receptor (**b**, lower panel, **c-e**). (**f-h**) FITC-labelled *S. aureus* were incubated with either mediun alone, anti-*S. aureus* mouse IgM or IgG mAbs. The *S. aureus* were then incubated overnight at 37^{o}C with mouse spleen cells pretreated with anti-mouse FcγRIII/II to block binding to Fc receptors for IgG. The spleen cells were then treated with 0.1% trypsin, stained with PE-conjugated anti-B220 mAb and analyzed by flow cytometry. Data are shown in the histogram gated for the B220^{+} B cell population (**f**). B220^{+} B lymphocytes

expressing green fluorescence were sorted and analyzed by using fluorescent microscopy under light (**g**) or dark field illumination (**h**). Overlapped pictures with green- and red-fluorescence are shown (**h**).