General introduction

I. INTRODUCTION

Human IgA is the prevalent antibody isotype involved in the defense against microorganisms, especially at mucosal surfaces. Binding of IgA-opsonized particles (e.g. bacteria, viruses) to myeloid cells bearing receptors for the Fc region of IgA (FcαR) may trigger various cell-mediated immune effector functions to eliminate foreign antigens.

FcαRI is the first Fc receptor for IgA that has been molecularly characterized. It represents a transmembrane glycoprotein that binds IgA1 and IgA2 with similar affinity. FcαRI is constitutively expressed on myeloid cells such as neutrophils, eosinophils and monocytes. Genetic characterization has shown that FcαRI is a distantly related member of the Ig receptor gene family. FcαRI has a short cytoplasmic tail without consensus signaling motifs. Similar to other receptors, which lack signaling motifs, FcαRI can associate with the FcR γ-chain signaling subunit through a charge-based mechanism. Upon IgA binding, triggering of FcαRI results in effector functions such as phagocytosis, induction of neutrophil degranulation, antibody dependent cell-mediated cytotoxicity (ADCC) and release of inflammatory mediators.

Since defects in IgA clearance have been proposed to play an important role in the pathogenesis of IgA-mediated diseases, studying the precise mechanisms of IgA binding to FcαR, in specific tissues or on selected cells in the human body is important.

II. IMMUNOGLOBULIN A

The IgA molecule is a tetramer, consisting of two identical light (κ or λ) and two heavy (α) chains (Figure 1). In humans, there are two isotypes of IgA, IgA1 and IgA2 *(1)*. In addition 3 allotypic variants of human IgA2 have been identified, designated A2m(1), A2m(2) and A2m(3) *(2)*. The α-chain contains 1 variable domain V_H and 3 constant domains Cα1, Cα2 and Cα3. In IgA1 there is a hinge region between Cα1 and Cα2, of which 13 aminoacids (aa) are not present in IgA2 *(1)*. This hinge region is susceptible to cleavage by specific bacterial IgA1 proteases. Furthermore, IgA1, in contrast to IgA2, possesses 5 O-linked carbohydrate moieties at serines in the hinge region *(3)* (Figure 2).

Serum IgA is predominantly monomeric in structure (mIgA), although dimers (dIgA) and polymers (pIgA) are present. To enable dimer Ig formation, IgA bears an additional 18 aa C terminal to the Cα3 domain, which similar to IgM monomers can interact with a joining (or J) chain. J chain is a small molecular weight glycoprotein (15kD) that contains 6 interchain disulfide bonds. In addition,

J chain bears 2 cysteine residues, each of which can form a disulfide bond with cysteine residues in the mIgA tail piece, thus stabilizing a dimeric molecule *(5,6)*.

In the mucosa dimeric IgA complexes are transported from the basolateral to the apical surface of mucosal epithelial cells, via the poly (Ig) receptor (pIgR). During this highly specific process, the extracellular part of the pIgR, which is covalently bound to one of the IgA monomers, is cleaved *(7)*.

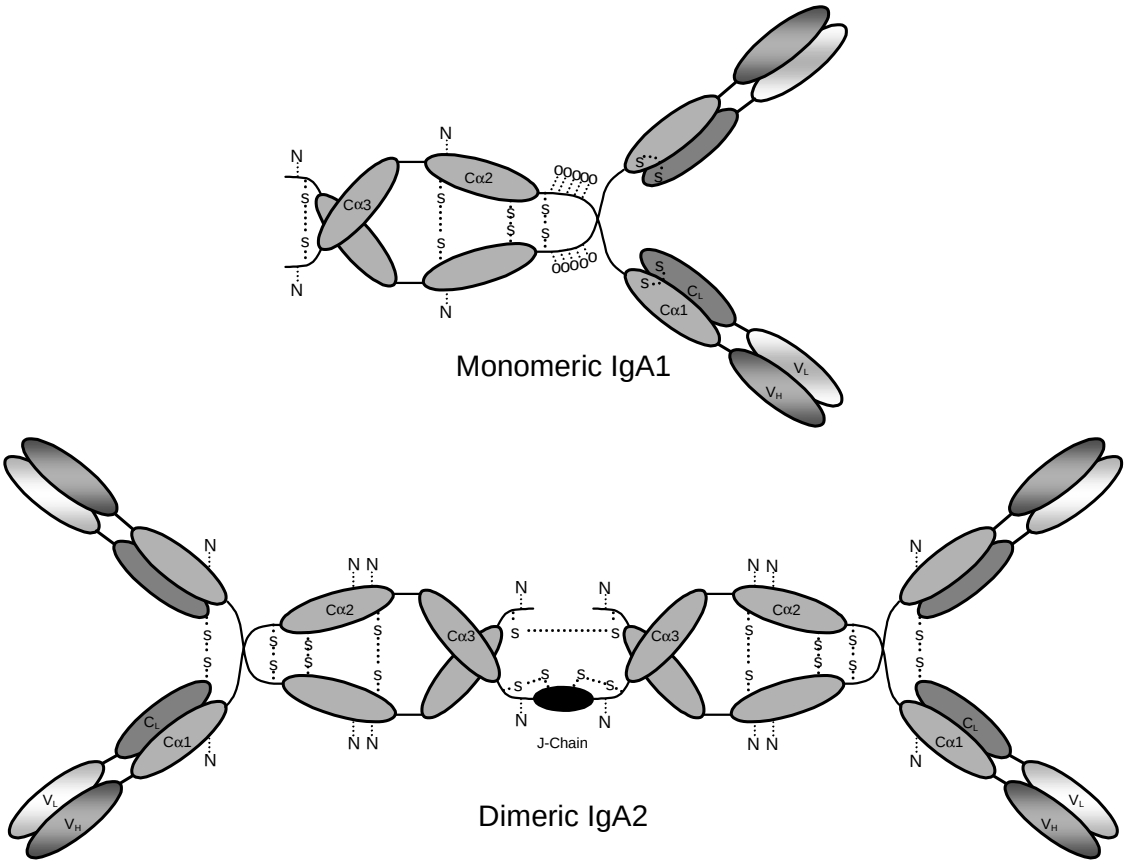

***Figure 1*. Proposed domain structure of human mIgA1 and dIgA2.** *The α heavy chain contains 1 variable domain V_H and 3 constant domains Cα1, Cα2 and Cα3. The light chain contains 1 variable domain V_L and 1 constant domain C_L. Positions of disulfide bonds (S), N-(N) and O-(O) linked sugars are indicated. (adapted from (4)).*

Therefore, the dimeric IgA molecule, upon secretion, acquires an additional peptide termed secretory component (SC), finally resulting in secretory IgA (sIgA) *(8)*. Secretory IgA serves as the first line of defense at mucosal surfaces. IgA polymerization can occur in the absence of J chain, as illustrated by the fact that J chain knock out mice have high concentrations of dIgA *(9)*. In contrast, in the absence of J chain, pIgR-mediated transport is hampered.

Protective antibodies of the IgA isotype have been described against a wide range of human pathogens including viruses such as HIV-1 and influenza, bacteria, bacterial toxins and parasites *(11)*. The mechanisms by which IgA exerts its protective effect are partially passive; this includes neutralization of viruses by blocking their receptors for host cell proteins (immune exclusion) and inhibition of bacterial motion by aggregation. However, when IgA attached to foreign antigen recruits effector systems such as complement, T cells and granulocytes, the protective effect of IgA is mediated actively and receptors for IgA are involved.

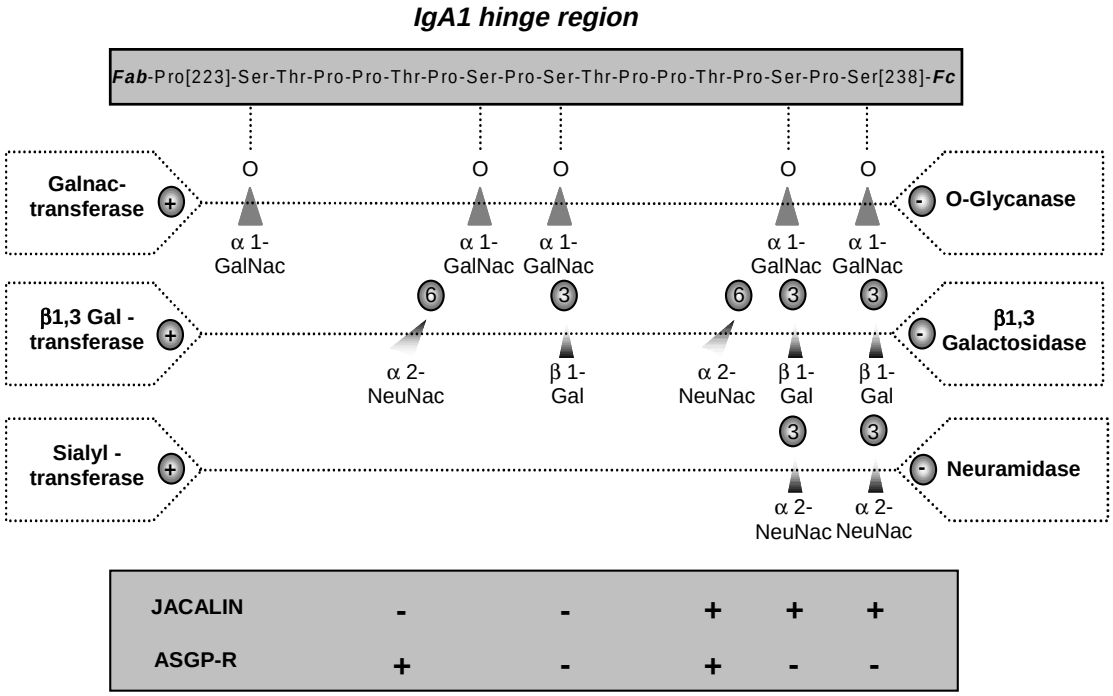

Figure 2. Schematic diagram depicting the hinge region of IgA1 from proline[223] upto serine[238] with the predicted O-linked glycan side chains, as indicated. *The numbers 3 and 6 indicate to which relative carbon atom of a glycan, the next glycan side chain is connected. Enzymes involved in glycosylation (left) and deglycosylation (right) are indicated. Depicted glycan side chains: GalNAc, N-acetyl galactosamine; NeuNAc, N-actelyl neuraminic (sialic) acid; Gal, galactose. Binding capacity to Jacalin, an IgA1 binding lectin and the ASGP-R are indicated at the bottom (adapted from (10)).*

In the liver, IgA clearance is mediated via an endocytotic mechanism involving the asioglycoprotein receptor (ASGP-R). The ASGP-R is a 46-kD single subunit receptor of the C-type (Ca^{2+}-dependent) lectin-family expressed on the surface of hepatocytes. These molecules recognize proteins via their oligosaccharide chains, specifically terminal galactosyl residues. The receptor is endocytosed and proteins bound to the receptor are targeted for degradation. The ASGP-R binds IgA1 via its carbohydrate side chains in the hinge region (Figure 2), targeting IgA for

clearance. In the circulation, binding of IgA is mediated via FcαRI, the first Fc receptor for IgA that has been molecularly characterized.

III. FC RECEPTORS

Receptors for the constant regions of all five classes of human immunoglobulins have been described *(12)*, but only Fc receptors for IgG and IgE have been studied intensively *(13,14)*. Three different types of IgG Fc receptors (FcγR), all members of the Ig superfamily, were identified on human blood cells. FcγRI (CD64), a 72-kD glycoprotein, is constitutively expressed on monocytes and macrophages and can be induced by IFNγ or G-CSF treatment on neutrophils. FcγRI serves as a high affinity (Ka ~ $5x10^8$M) receptor for monomeric IgG. In contrast, FcγRII (CD32), a 40kD glycoprotein found on a wide variety of cell types, including monocytes, platelets, neutrophils and B cells, is a low affinity (Ka ~ 10^6M) receptor. Finally, FcγRIII (CD16) is a variably glycosylated, low affinity receptor (Ka ~ 10^6M) of 50-70 kD, that is present on neutrophils, NK cells, eosinophils, a subset of T cells and cultured monocytes. FcγRIII binds poorly to monomeric IgG but interacts well with aggregates or complexes of IgG. On neutrophils FcγRIII is anchored in the membrane via a glycosyl-phosphatidylinositol (GPI) linkage, whereas on NK cells a transmembrane form is present *(13,15)*.

For IgE, two types of Fc receptors have been described. The high affinity FcεRI, also a member of the Ig superfamily, is composed of a 45-kD α-chain, and a 33-kD β-chain, and expressed on mast cells and basophils. It has furthermore been detected on monocytes, eosinophils and Langerhans cells of selected individuals *(16)*. The low affinity FcεRII (CD23), a 45-kD glycoprotein, is present on monocytes, eosinophils, T cells, B cells and activated macrophages. This receptor however belongs to the C-type lectin superfamily *(14)*.

Although receptors for IgA have been recognized since the early 1980's *(17-20)*, relatively little is known about their nature and function. IgA binds to various types of blood cells, such as human neutrophils, monocytes, macrophages, eosinophils, B cells, T cells and myeloid cell lines *(21,22)*. Evidence for existence of FcαR on a subpopulation of human B-lymphocytes was obtained in a rosetting assay with mouse IgA-coated on ox RBC. FcαR was shown to be present on 5-20 % of B cells from peripheral blood of normal volunteer's *(18)*. Rabbit IgA-coated sheep RBC were used in a rosetting technique to demonstrate the expression of a FcαR on human T lymphocytes *(19)*. Peripheral human neutrophils and monocytes were shown to form rosettes with human IgA1 or IgA2 coated ox RBC *(20)*. Interestingly, in vitro experiments with murine T cells demonstrated that they could form rosettes with human IgA1-coated ox erythrocytes. It was found that

both human IgD and IgA1 bind via their O-linked glycans to the murine IgD receptor *(23)*.

At present, an IgA Fc receptor found on monocytes, neutrophils and eosinophils (FcαRI), is the only FcαR that has been defined molecularly *(24)*. This myeloid FcαRI was given the designation CD89 at the Fifth leukocyte-typing workshop *(25)*.

IV. FCαRI/CD89

IV.1. Genetics

A 1.6-kb cDNA clone encoding CD89 was isolated from PMA-stimulated U937 cells, using the CD89 mAb My43 *(26)* and expression cloning in COS-7 cells *(24)*. The CD89 gene contains 5 exons and 4 introns *(27)*. The first exon or S1, encodes for the 5'-untranslated region, the ATG start codon and a 27 bp stretch of the coding sequence for the leader peptide. Exon 2 or S2, is 36 bp long and encodes the remaining part of the leader peptide, including the predicted signal peptidase cleavage site between the alanine-21 and glutamine-22 residues. The third and fourth exons encode the extracellular domains one (EC1) and two (EC2), respectively. The fifth exon encodes a short extracellular segment, the transmembrane domain and a short cytoplasmic tail with a TAA stop codon *(27)*. More recently, a sixth exon was described *(28)*. However, this 78 bp exon or S3, between S2 and EC1, encodes a stop codon, and is thus believed to represent a pseudo-exon *(29)*.

All FcγR genes (in man, rats and mice), as well as FcεRI, have a 21 bp S2 mini exon, instead of the 36 bp S2 exon in CD89 *(12)*. Interestingly, only in bovine Fcγ2R has such a 36 bp S2 mini exon has been found, suggesting that this bovine IgG receptor is more closely related to CD89 compared to the other human FcR genes *(30)*.

The CD89 gene has been localized on chromosome 19, at position 19q13.4 *(31)*. This is different from all other Fc receptor genes, which are located on chromosome 1. However, the 19q13.4 region does contain multiple genes encoding type I transmembrane molecules, all of which belong to the Ig-superfamily, termed the leukocyte receptor complex (LRC) *(32,33)*. This family includes the killer inhibitory receptors (KIR's) *(34,35)*, the Ig-like transcript receptors (ILT) *(36)*, leukocyte Ig-like receptors (LIR), Monocyte/Macrophage Ig-like receptors (MIR) *(37)* and the leukocyte-associated immunoglobulin-like receptor (LAIR) *(38)*. On the basis of homology with CD89, the paired Ig-like receptors (PIR-A and PIR-B) *(39,40)* have been identified in mice. Their genes are

located on mouse chromosome 7 in a region syntenic with human chromosome 19q13.

IV.2. Protein structure

The CD89 protein has a 21 aa leader peptide. This hydrophobic leader peptide is cleaved between position Ala21-Glu22 during membrane insertion, resulting in the formation of a 266 aa mature type I transmembrane glycoprotein. The 206 aa extracellular part of CD89 consists of two Ig-like domains and carries one potential O-linked, and 6 potential N-linked glycosylation sites. The transmembrane part is 19 aa long and bears a positively charged arginine (Arg 209) which is essential for association of CD89 with the FcR γ-chain homodimer-signaling subunit *(41,42)*. The short cytoplasmic tail of 41 aa of CD89 contains no consensus signaling motifs of its own (Figure 3).

The predicted molecular weight of CD89 is 30 kD *(24)*. The apparent molecular weight of CD89 on monocytes and neutrophils is 55-75 kD *(43)* and on eosinophils 70-100 kD *(44)*, due to heterogeneous glycosylation. Deglycosylation of CD89 from eosinophils and neutrophils with N-glycanase gives rise to distinct bands of 32 and 36 kD in size *(43,44)*. Deglycosylation with neuramidase and O-glycanase together with N-glycanase on U937 cells, a CD89 expressing monocytic cell line, revealed two distinct bands of 32 and 34 kD *(45)*. Finally, tunicamycin treatment of U937, which induces complete inhibition of N-linked glycosylation, followed by CD89 specific immunoprecipitation revealed only one band of 32 kD *(45)*. These experiments point at the complexity in CD89 glycosylation between various myeloid cell types.

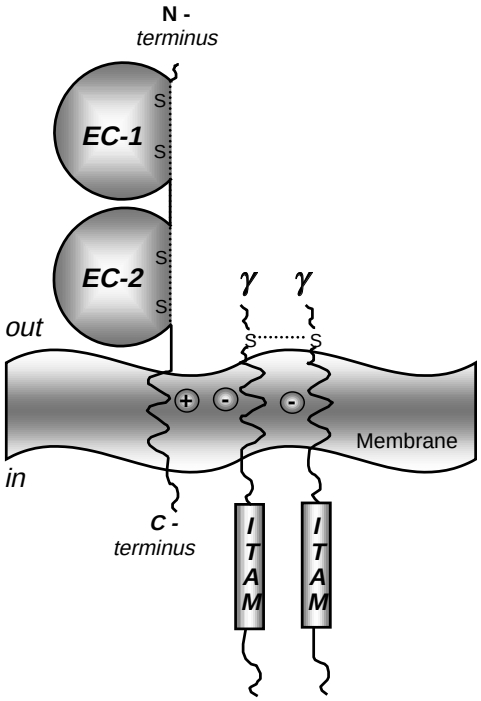

Figure 3. Structure of the CD89 FcR γ-chain complex. *The extracellular domain 1 and 2 (EC-1 and EC-2), as well as the γ-chain with its signaling (ITAM) motifs and the charged residues responsible for association are depicted.*

The IgA protein has been studied to find the specific domains that are involved in CD89 binding. Using both domain-swapped IgA/IgG and point-mutated IgA chi-

meras, expressed by recombinant baculovirus infected cells, the CD89 recognition site on IgA was located *(46)*. It was demonstrated that neither the hinge region, nor the tailpiece was necessary for binding. When mutations were made near the boundary between Cα2 and Cα3, binding to CD89 was ablated. Recent studies showed in more detail that the Leu^{257}-Gly^{259} region in Cα2 and Pro^{440}-Phe^{443} region in Cα3, are crucial for CD89 binding and triggering, thereby enhancing present understanding of the molecular basis of the IgA-CD89 interaction *(47)*.

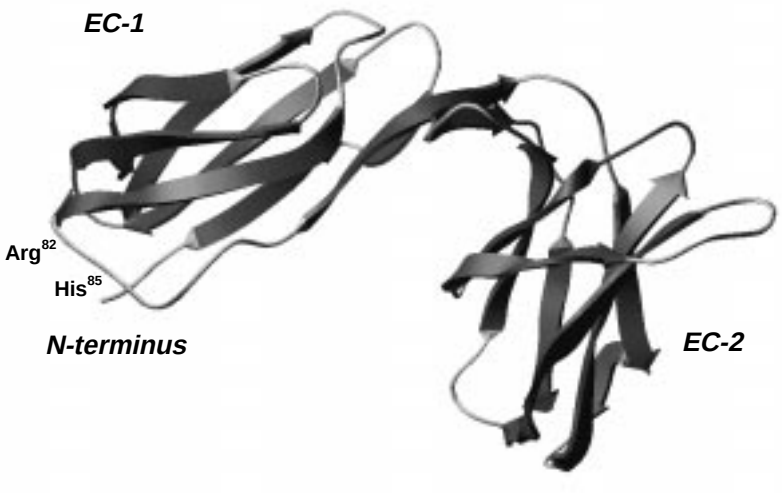

Figure 4. A model of the 3 dimensional structure of the extracellular part of CD89, based on homology with the KIR structure. *The EC-1 and EC-2 domains, N-terminus, Arginyl 82 and Histidyl 85 residues are depicted.*

The IgM mAb My43 blocks binding of IgA to CD89, whereas all other known CD89 mAb, A3, A59, A62, and A77 (all of the IgG1 isotype) do not *(26,45)*. With a soluble form of CD89 and a strategy combining chemical modification and site-directed mutagenesis, the histidyl 85 and arginyl 82 residues in the EC1 domain of FcαRI were shown to be essential for binding of IgA *(48)*. On the basis of homology with the KIR sequence and the crystal structure, a model for CD89 has been made *(49,50)* (Figure 4). It was demonstrated that CD89, like several IgG receptors, represents a low affinity receptor for IgA (Ka ~ 10^6 M^{-1}). Rapid dissociation of the soluble form of CD89 complexed with IgA ($t_{1/2}$ ~ 25 s) suggests that mIgA binds transiently to cell bound CD89, while pIgA or IgA immune complexes (IgA-IC) may bind more avidly.

IV.3. Splice variants

A number of alternatively spliced CD89 mRNA's have been found in cDNA libraries of CD89-expressing cells (Figure 5). Three variants identified in neutrophils lacked coding sequences from the exons S2, EC2 or S2 plus EC1. Two other variants lacked 66 bp from EC2, due to the use of an alternative splicing site 66 bp from the 3' end. In one of these latter cDNAs, S2 was also deleted *(51)*.

The variant lacking EC2 was identified in neutrophils, eosinophils, alveolar macrophages THP-1, and U937 cells *(52-54)*.

Recently a FcαR variant has been cloned from a human eosinophil cDNA library (FcαRb), which shows an abberant transmembrane/intracellular region *(55)*. In alveolar macrophages, two alternatively spliced products corresponding to deletions of 66 and 288 bp in the EC2 domain were detected, FcαRa.2, and a.3, respectively *(52)*. The FcαRa.2 molecule was detected on the membrane of alveolar macrophages, and N-glycanase treatment resulted in a protein core, smaller to that on the FcαRI backbone of blood monocytes *(52)*.

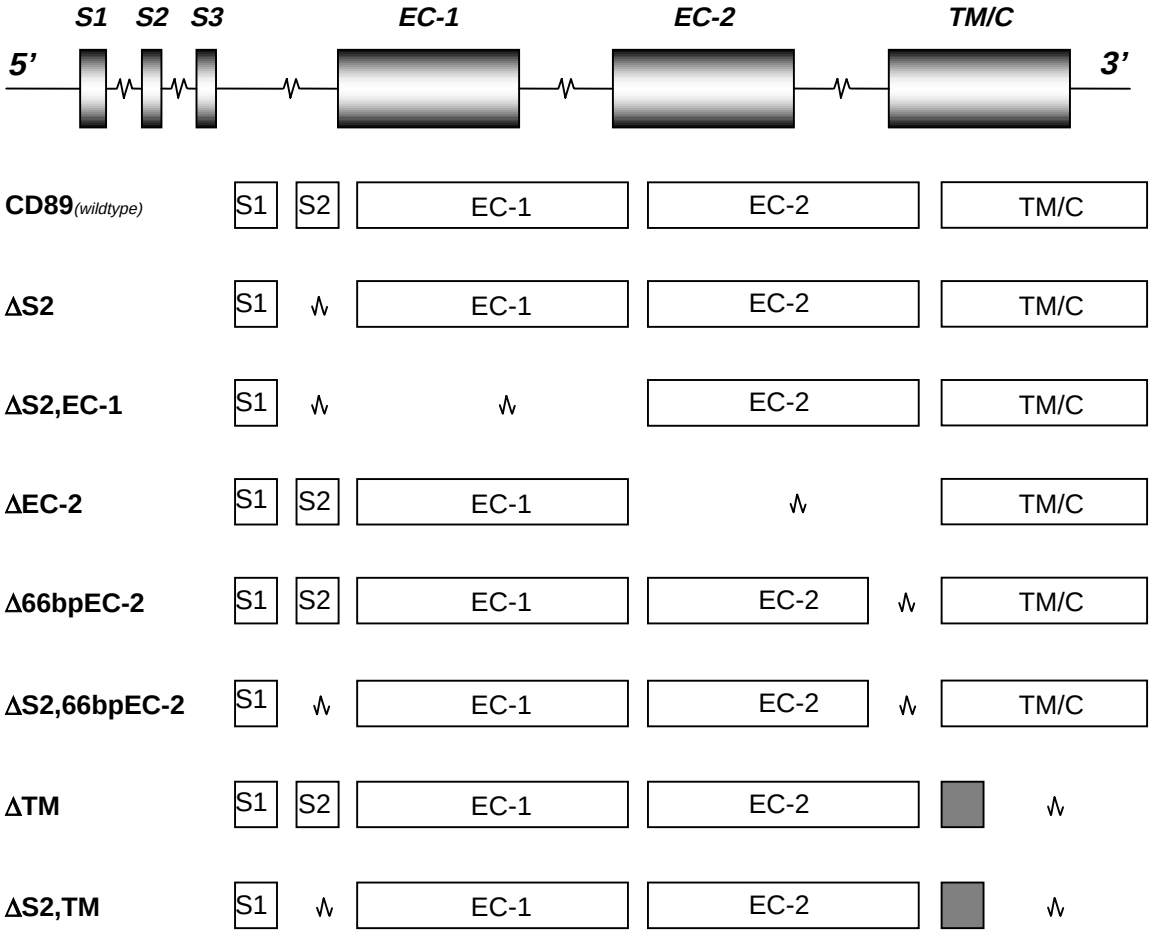

Figure 5. Schematic view of the different splice variants that have been described for CD89. *From top to bottom: The exon organization derived from the gene map (not on scale), CD89 (wildtype) (27), CD89/ΔS2, CD89/ΔS2,EC-1 (51), CD89/ΔEC-2 (or FcαRa.3) (51-54), CD89/Δ66bpEC-2 (or FcαRa.2) (51,52), CD89/ΔS2,66bpEC-2 (51), ΔTM (or FcαRb) and ΔS2,TM (or FcαRbΔS2) (55).*

The biological relevance of the various FcαRI splice-variants still remains unclear *(52)*. In view of the fact that splice-variants of certain FcγR and FcεR have been

found to be expressed *in vivo*, as biologically active receptors *(13)*, the FcαRI splice variants may have physiologic relevance.

Next to alternative splicing, membrane bound receptors can be released via proteolytic cleavage, as demonstrated for various molecules, including cytokines, cytokine receptors, adhesion molecules and Fc receptors *(56-58)*. Soluble forms have been identified for Fc receptors for IgG (FcγRII/CD32, and FcγRIII/CD16) and IgE (FcεRII/CD23) *(59-61)*. Release of soluble receptors has been suggested to represent an universal mechanism of receptor regulation, which might be dysregulated in various human diseases *(56)*. Levels of sCD16 (FcγRIII) have been proposed to be a measure for the number of neutrophils present in the circulation *(60)*. Little or no information is available on the existence of soluble forms of FcαR.

IV.4. Cell distribution and modulation of expression

Using Northern blot analysis (with parts of the CD89 cDNA as a probe), CD89 mRNA was detected in monocytes, neutrophils and eosinophils *(24,43)*. FACS analysis using CD89 mAb, demonstrated the presence of CD89 at the surface of myeloid cells such as monocytes, neutrophils, macrophages and eosinophils. It has been estimated that both monocytes and neutrophils express between 6,000 and 7,000 CD89 molecules per cell *(62,63)*. In addition, myeloid cell lines such as U937, THP-1, MonoMac-6, HL-60 and PBL985 express CD89 at comparable levels.

Both Northern blots and FACS analysis revealed that human T cells, B cells, NK cells, platelets, erythrocytes, B cell lines or K562 cells are negative for CD89 *(21,24,26,45)*. The expression of CD89 by human kidney mesangial cells remains controversial, although some results from Northern blotting have supported the presence of CD89 in rat and human mesangial cells *(64)*. CD89 membrane expression, however, was not detectable using antibodies on human mesangial cells, and IgA induced activation of human mesangial cells was shown to be independent of CD89 *(65)*.

Some early studies demonstrated that the expression of myeloid FcαR on peripheral blood neutrophils could be enhanced by overnight incubation with IgA *(20,43)*. In addition to an IgA-induced increase in CD89 expression *(22)*, different cytokines and other factors (Figure 6) can modulate the number of CD89 molecules.

CD89 expression on monocytes can be increased by incubation with IL-1β, TNFα, GM-CSF, LPS, PMA, and the differentiating agent calcitriol *(66-69)*. In contrast, incubation with TGFβ, or the polyanionic compound suramin reduce CD89

expression levels *(70,71)*. CD89 expression levels on the monocytic cell lines HL-60, PBL985, THP-1, MonoMac-6 and U937 can be modulated in a similar manner to that on monocytes, with the exception of CD89 expression on MonoMac-6, which remains unaffected after GM-CSF, or IL-1β treatment *(66,67)*.

Neutrophils and eosinophils, stimulated with chemotactic agents such as fMLP and zymosan-activated-serum (ZAS, a source of C5a), upregulate CD89 expression in a Ca^{2+}-dependent manner *(77)*. Interestingly, upregulation of CD89 membrane expression on U937 appears to be Ca^{2+} independent *(44)*. Furthermore, the rapid (within 20 minutes) CD89 upregulation found in neutrophils is not affected by inhibition of protein synthesis, suggesting the existence of an intracellular pool of CD89 *(77)*. Also, IL-8 and TNFα upregulate CD89 expression on neutrophils, whereas GM-CSF has no effect *(44,72,76)*.

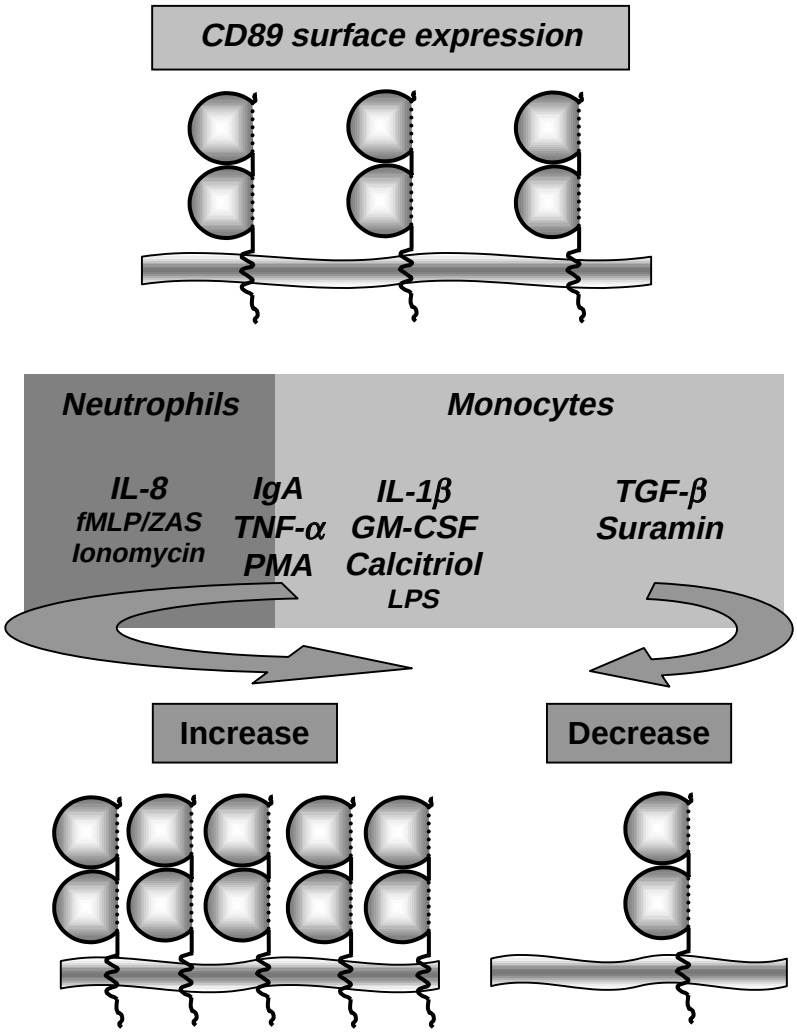

Figure 6: Modulation of CD89 surface expression*. CD89 surface expression increases after treatment with IL-8 (76), fMLP/ZAS (77) and ionomycin (77) on neutrophils only; with IgA (22), TNFα (66,67,72,73), and PMA (24,43,45,68) on both, monocytes and neutrophils; and with IL-1β (67), GM-CSF (67,74), Calcitriol (69,74,75), and LPS (67) on monocytes only. CD89 surface expression decreases after treatment with TGFβ (70) and suramin (71) on monocytes.*

IV.5. Signal transduction

Although CD89 has a short cytoplasmic tail, one without consensus signaling motifs, crosslinking of the receptor induces intracellular signaling and leads to cell

activation *(62,78)*. Like other leukocyte Fc receptors that lack intracellular signaling motifs, intracellular signals are initiated via association of CD89 with a small (10 kD), predominantly intracellular, dimeric γ-chain. The Fc receptor γ-chain was first identified as a subunit of the FcεRI complex *(79)*. Subsequently it was shown to associate with members of all three classes of human leukocyte FcγR, as well as with TCR-CD3 complexes, where the γ-chain is responsible for coupling of receptors to intracellular signaling pathways *(12,80,81)*. A functional association between the γ-chain homodimer and CD89 was observed only when a positively charged aa was present within its transmembrane domain. Substitution of Arg209 with another positively charged aa preserved association with the γ-chain homodimer, whereas substitution with a neutral or negatively charged aa did not *(42)*.

In addition to acting as a signal-transducing subunit, it has been documented that γ-chain may enhance FcγRI ligand-binding affinity *(82)*. Furthermore, the FcR γ-chain may play a role in stabilizing cell surface expression of receptors. For example, FcεRI, FcγRI and FcγRIIIa all require association with FcR γ-chain for stable surface expression *(79,83)*. Recently it was demonstrated that CD89 surface expression was absent when CD89 transgenic mice were crossed with γ-chain knock-out mice *(73,84)*. However, expression of CD89 in IIA1.6 cells appears independent of FcR γ-chain co-transfection *(42,85)*. At present it is not clear how this presumed discrepancy can be explained.

Crosslinking of CD89 results in the phosphorylation of ITAM motifs on the γ-chain. Src family phosphotyrosine kinases (PTKs) are responsible for this phosphorylation and kinases thought to be critical include p56Lyn *(86)*, Syk and Bruton tyrosine kinase (Btk)*(87)*. Following ITAM phosphorylation, a Syk family member, p72syk or ZAP-70 is recruited to the complex *(86)*, resulting in the phosphorylation/activation of further downstream proteins, including PKC, PLCγ and MAP kinases *(88)*, ultimately triggering cellular effector functions.

IV.6. CD89 functions

Phagocytosis of IgA complexed antigens represents an important effector function of CD89 (Figure 7). First evidence for FcαR-mediated phagocytosis was found in monocytes, by inhibition studies of IgA coated erythrocytes uptake using the CD89 mAb My43 *(26)*. Before the molecular characterization of CD89, it was shown that unstimulated neutrophils phagocytose both yeast coated with serum IgA anti-mannan antibodies and *S. aureus* opsonized with serum or secretory IgA, in a FcαR dependent fashion *(89)*. IgA-opsonized latex particles were inefficiently phagocytosed by unstimulated PMN. These cells needed priming with GM-CSF or

IL-8 for enhanced uptake. The increased phagocytosis correlated with higher membrane expression of the CD89 molecule results from the GM-CSF or IL-8 priming *(76,90)*. Similarly, priming of monocytes with IL-1β, TNFα, GM-CSF, and LPS, upregulates CD89 membrane expression and enhances IgA-mediated phagocytosis *(67)*.

Chemically aggregated IgG and IgA are able to induce neutrophil degranulation *(91)*, and these Fc-receptor mediated processes could be primed by fMLP *(77)*. In addition, non-aggregated serum IgA1 and IgA2 trigged degranulation, similar to both dimeric IgA and My43 after crosslinking CD89 *(92)*. Neutrophil degranulation for a given concentration of IgA was greater than that of similar concentrations of IgG *(91)*. On monocytes, the induction of superoxide release triggered by either serum IgA or secretory IgA and IgG was equal. Since CD89 expression on monocytes was lower as compared to FcγR's, these experiments suggest that CD89 triggering results in higher activation levels when compared to other FcγR's *(26)*. In addition, aggregated IgA has been shown to degranulate eosinophils and to release eosinophil derived neurotoxin *(93)*.
Antibody dependent cell-mediated cytotoxicity (ADCC), is another important effector function of FcαR. Crosslinking FcαR's on monocytes and neutrophils with IgA can trigger ADCC *(94,97)*, and IgA2 opsonization of *Schistosoma mansoni* results in effective killing of this parasite *(109)*. Furthermore, monocytes mediate an IgA dependent killing of bacteria from patients with meningococcal infections *(98)*. Similarly, mononuclear cells efficiently lyse erythrocytes coated with IgA, isolated from patients suffering from acquired immune hemolytic anemia in a complement-independent, IgA-dependent manner *(99)*.

Release of cytokines following FcαR stimulation is an important function that can result in recruitment of other effector cells to sites of infection. Crosslinking monocytic CD89 with specific mAb, followed by Fab'2 fragments of an anti-mouse antiserum has been shown to induce release of a number of cytokines such as TNFα, IL-1β and IL-6 *(100,110)*. Aggregated IgA also has been shown to trigger release of inflammatory mediators such as leukotrienes C4 and B4 and prostaglandin E2 from monocytes *(102)*.

Recently, bispecific molecules were produced by chemical crosslinking of F(ab') fragments of the CD89 mAb A77, with a tumor Ag-specific mAb and examined for their ability to mediate ADCC and phagocytosis. These BsAb effectively mediate ADCC and phagocytosis of tumor cells by monocytes and neutrophils in a CD89 dependent manner *(111)*. Furthermore, similar bispecific molecules directed to CD89 and *Candida albicans*, enhanced neutrophil phagocytosis of fungi *(112)*. Comparison between CD89, FcγR's and CR3 as

trigger molecules, showed CD89 mediated ADCC and phagocytosis to be most efficient *(113)*.

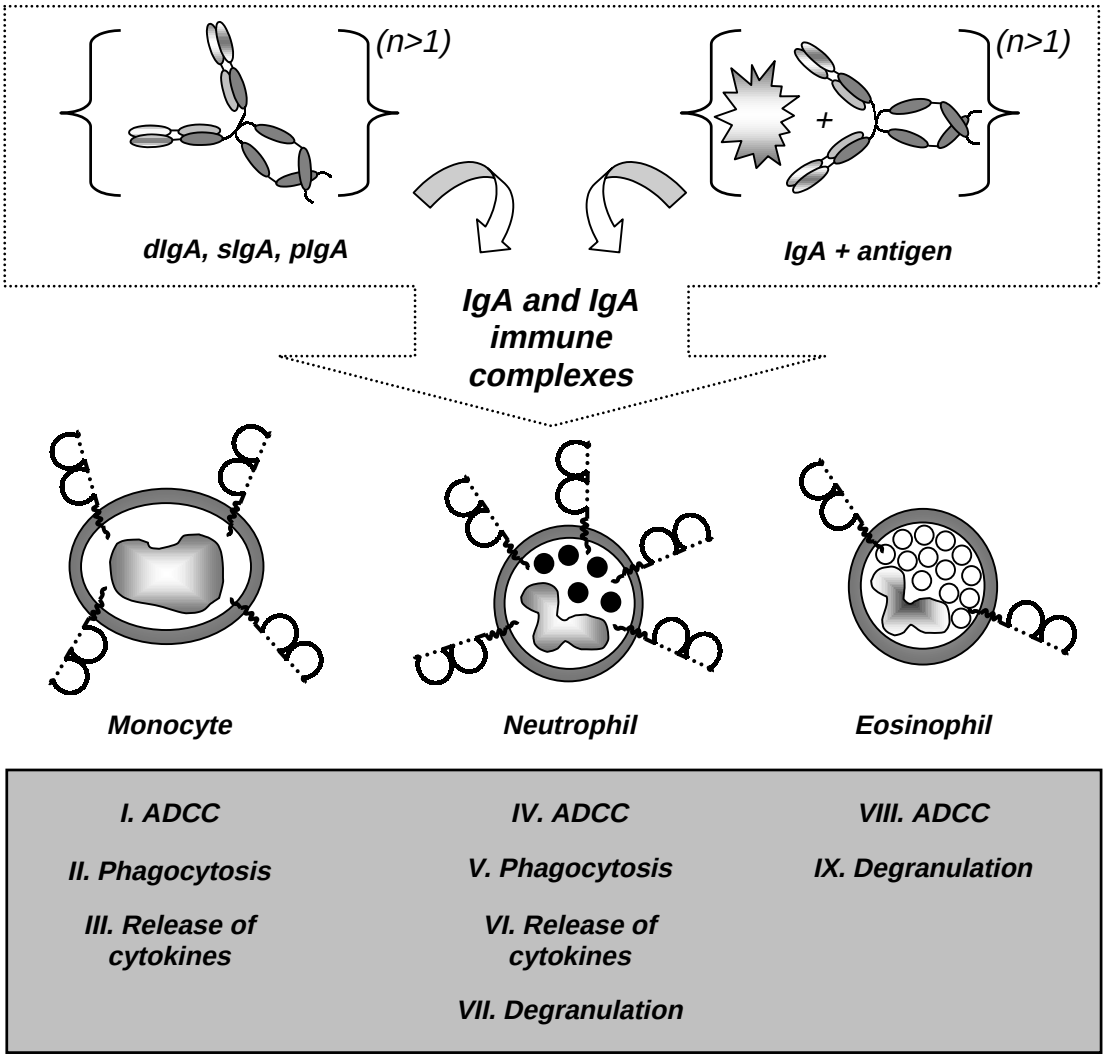

Figure 7: *Summary of the described functions for FcαR, CD89 expressed on the surface of* ***Monocytes: I.*** *ADCC (94-99),* ***II.*** *Phagocytosis (26,62,67,95),* ***III.*** *Release of TNFα and IL-6 (increased) (100), (decreased) (101), IL-RA (86,101), IL-1α (100), IL-8 (86), leukotrienes C4, B4 and prostaglandin E2 (102);* ***Neutrophils: IV.*** *ADCC (94,96,97),* ***V.*** *Phagocytosis (62,76,90,103-106),* ***VI.*** *Release of TNFα (72),* ***VII.*** *Degranulation (26,67,77,78,91,92,103,107,108) and* ***Eosinophils: VIII.*** *ADCC (96,109),* ***IX.*** *Degranulation (88,93).*

Phagocytic IgA receptors have been proposed to function in the clearance of IgA-antigen complexes from the blood *(114)*. IgA from serum is efficiently internalized by PMN, whereas CD89 mAb need to be crosslinked before internalization by PMN, monocytes, and transfected IIA1.6 cells *(42)*. Therefore, CD89 may mediate the removal of IgA, and small IgA-complexes from the circulation in normal individuals.

V. DISEASES ASSOCIATED WITH IGA AND CD89

Defective CD89-mediated endocytosis has been suggested to be relevant for several IgA-associated diseases, including HIV-1 infection, alcoholic liver cirrhosis (ALC) and IgA-Nephropathy (IgAN) *(115-118)*. All of these diseases have elevated serum IgA concentrations and increased levels of IgA-containing immune complexes, when compared to healthy controls.

Primary IgA nephropathy (IgAN) is the most common form of primary glomerulonephritis, with a broad spectrum of clinical presentations, leading to progressive renal failure in a substantial proportion of patients *(119,120)*. The disease is characterized by increased production of plasma IgA1 by the bone marrow and by deposits of IgA1 in the glomerular mesangium *(120,121)*. The mesangial IgA has been found to consist of pIgA *(122)*. The mechanisms responsible for the mesangial depositions remain unclear *(120)*. It has been hypothesized that a receptor for IgA can be expressed on mesangium, and thereby is involved in IgA deposition. In accordance with this hypothesis, it was suggested that mesangial cells *in vitro* express CD89 mRNA, however membrane expression was not investigated *(64)*. Polymeric IgA and dIgA, but not mIgA bind efficiently to mesangial cells and enhance IL-6 expression levels *(123)*. In addition both pIgA and mIgA bound to human mesangial cells independent of CD89 and only pIgA induced c-jun expression, suggesting the presence of a new FcαR on human mesangial cells *(65)*.

It has been suggested that phagocytes in IgAN patients were "over-saturated" with IgA, and are defective in CD89-mediated endocytosis *(115)*. Other IgA mediated effector functions like ADCC, superoxide generation and release of cytokines and inflammatory mediators have been linked to tissue damage *(21)*. Thus a defect in CD89-mediated clearance of IgA immune complexes is thought to contribute to the pathogenesis of these diseases *(124)*.

The quantitative difference in serum IgA concentrations between patients with IgAN and controls seems to be caused by a increased production of IgA1 by the bone marrow *(120)*. However, a deficient mucosal IgA immune response has been reported in patients with IgAN *(125)*. In addition to increased production, qualitative differences in the IgA molecule itself, may also influence its clearance and deposition. IgA contains 2 to 14% by weight of glycans linked to the immunoglobulin heavy chains. Recently it was suggested that the O-linked carbohydrates in the hinge region of the IgA1 molecule contain less galactose (Gal) *(126,127)*. Glycans on glycoproteins and glycolipids play an essential role in many biological functions, such as homing of lymphocytes to various tissues, binding of Ig to cellular receptors, phagocytosis, activation of the complement cascade, and

binding, internalization, and catabolism of Ig by hepatocytes *(128)*. An altered IgA1 glycosylation could cause decreased binding to FcαR and results in defective clearance of IgA and IgA-immune complexes, explaining the observed higher serum IgA1 levels and or IgA1 deposition. In addition, down regulation of CD89 on monocytes of patients with IgAN was demonstrated, indicating that aberrant regulation of CD89 may also contribute to higher serum IgA levels *(129)*.

VI. SCOPE OF THIS THESIS

For a better understanding of the role of IgA in immunity, we focussed on the interaction between the prototypic IgA receptor CD89 and different forms of IgA. We developed a set of specific reagents for further analysis of IgA-CD89 interactions, and localized the immunoglobulin-binding region on CD89 to the membrane-distal EC-1 domain (Chapter 2). Activation of the CD89 molecule resulted in an FcR γ-chain dependent shedding of soluble CD89 (Chapter 3).

In IgA-mediated diseases, deposition of IgA-complexes plays an important pathogenic role. We studied the binding of different sizes of IgA to CD89-transfected cells and found larger complexes of IgA to exhibit better binding to CD89 compared to mIgA (Chapter 4). A possible role for IgA receptors in IgAN was investigated, and resulted in documentation of reduced binding of immunoglobulin A (IgA) from patients with primary IgA nephropathy to CD89 (Chapter 5). Finally, we demonstrated that human mesangial cells in culture and in kidney sections lack CD89 expression (Chapter 6), suggesting the presence of an alternative IgA receptor in the kidney.

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