

Autoantibodies, human Fc γ receptors, and autoimmunity

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Abstract: Receptors for the Fc fragment of immunoglobulin G (Fc γ Rs) represent the link between the humoral and cellular immune responses. In humans, three different types of Fc γ Rs belonging to the immunoglobulin gene superfamily, have been identified; Fc γ RI (cluster of differentiation (CD64), Fc γ RII (CD32) and Fc γ RIII (CD16). Fc γ Rs are important molecules not only to mediate and control the effectors functions of immunoglobulin G antibodies, but they also control the autoimmunity–tolerance balance in the periphery. The development of autoimmune diseases is complex and dependent on multiple genes and environmental factors. A wide range of inflammatory and autoimmune diseases such as vasculitis, glomerulonephritis, and autoimmune hemolytic anemia, seems to be mediated, in part, by Fc γ Rs. Considering that the autoantibodies target intracellularly located antigens, recent findings supposed that, under certain conditions, Fc γ Rs are involved in the penetration of antibodies into cells and Fc γ Rs constitute one of the main effector mechanisms through which autoantibodies exert their action. In this review we concentrate on the role of human Fc γ Rs in autoantibodies penetration and summarize the current knowledge on the structure, ligand-binding capacity, and their role in autoimmunity and pathogenic effect of autoantibodies. These novel insights into antibody FcR interactions might be useful to produce the next generation of improved immunotherapeutic molecules.

Keywords: autoantibodies, Fc γ Receptors, IgG, adalimumab, salivary gland

Autoantibodies in autoimmune diseases: the predictive value of autoantibodies

Sometimes the immune system's recognition apparatus breaks down, and the body begins to manufacture antibodies and T cells directed against the body's own constituent-cells, cell components, or specific organs. Such antibodies are known as autoantibodies, and the diseases they produce are called autoimmune diseases. Many autoimmune diseases are chronic conditions that progress over the course of years and are characterized by the presence of serum autoantibodies, measured by immunoenzymatic test, that precede the overt disease by months or years. The presence of specific autoantibodies constitutes an important criterion supporting the clinical diagnosis of many organ-specific autoimmune diseases. The natural history of autoimmune diseases reveals that before the disease clinically manifests with signs and symptoms that lead to a definite diagnosis, disease onset is preceded by an asymptomatic phase which can last for many years and in which specific autoantibodies are already present in patients's sera.^{1,2} For this reason, a new piece in the mosaic of autoimmunity has clearly emerged in recent years, namely the predictive value of autoantibodies. Indeed, many autoantibodies can be detected in the preclinical phase of autoimmune diseases many years before

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the disease becomes apparent; furthermore, they have a high diagnostic positive predictive value (PPV).³

In systemic lupus erythematosus (SLE), a chronic autoimmune inflammatory disease with several internal organs involved, antibodies to ribonucleoprotein (RNP), SMITH (Sm) antigen, double stranded DNA (dsDNA), cardiolipin, nuclear protein Ro (Sjögren's syndrome type A, SS-A) and La (Sjögren's syndrome type B, SS-B) antigens have a PPV of 94%–100%. According to the type of antibody, the appearance can precede clinical diagnosis by 7–10 years with a frequency that varies from 32% to 78% at the moment of the diagnosis.²

In subjects with scleroderma, a progressive disease that affects the skin and connective tissue, anticentromere and antitopoisomerase I antibodies are detectable, with a PPV of 100%, up to 11 years before clinical manifestations. In rheumatoid arthritis (RA), an autoimmune disease which causes chronic inflammation of the joints, the tissue around the joints, as well as other organs in the body, the rheumatoid factor (antibody against the Fc portion of immunoglobulin G [IgG]), has a predictivity of 52%–88%, while for anticyclic citrullinated peptide (CCP; a circular peptide containing the amino acid citrulline) antibodies (anti-CCP) the predictivity is much higher, reaching 97%. If the rheumatoid factor and anti-CCP antibodies are both present, PPV rises to 100%. These two antibodies have even been detected in the serum up to 14 years before patients manifested the first symptoms of the disease.⁴

In Sjögren's syndrome (SS), an autoimmune rheumatic disease that targets salivary and lachrymal glands, anti-Ro and anti-La characterizing antibodies, were detected on average five years before the appearance of overt clinical signs and symptoms in 73% of asymptomatic mothers who had given birth to a child with autoantibody-associated congenital heart block and who later developed SS.⁴

Antinucleosome antibodies were found in 67% of patients with primary antiphospholipid syndrome, an immune disorder characterized by abnormal antibodies directed against phospholipids, up to 11 years before the development of SLE. Their PPV was 100%.⁴

Additionally, anticardiolipin antibodies may help to predict cases of SLE shifting to secondary antiphospholipid syndrome.⁵

In organ-specific autoimmune diseases – such as primary biliary cirrhosis, Addison's disease, a rare endocrine disorder in which the adrenal glands do not produce enough steroid hormones, Hashimoto's thyroiditis, thyroid gland autoimmune disorder, type 1 diabetes, celiac disease and Crohn's disease,

an inflammatory disease of the intestines – the predictive value of each antibody characteristic for a specific disease is similar to that for the autoantibodies in autoimmune rheumatic diseases.⁶

In summary, it is now clear that many autoantibodies have the ability to predict the development of an autoimmune disease in asymptomatic persons. It is also clear that the progression towards a given autoimmune disease, and its severity, can be predicted from the type of antibody, the antibody level, and the number of antibodies present.

The PPV of autoantibodies could be used to prevent the disease treating aggressively the patients prior to manifestations of symptoms. However criteria would have to be formalized for selection of patients for this preventive treatment. Only patients whose probability to develop clinical disease is higher than a certain threshold should be treated while asymptomatic.

The role of autoantibodies

Antibodies are naturally potent inducers of inflammation. It is not surprising then that the identification of autoantibodies in sites of inflammation has historically raised the question about such antibodies being primary mediators of the inflammation, or even central to the cause of the disease. Curiously, autoantibodies have often been seen as secondary products of the disease process. Early clinical immunologists were able to show the presence of immune complexes or autoantibodies in inflamed tissues, such as the kidney in nephritis, the blood vessels in vasculitis and joints in RA. Certainly in some diseases autoantibodies have been clearly identified as the primary cause of morbidity, for example, in SLE or immune thrombocytopenia purpura, whereas they have little if any role in others, for example, autoimmune type I diabetes. However, and interestingly, in other more complex diseases such as RA, the role of autoantibodies has been dogged by controversy. Early studies showing the presence of abundant IgG-reactive rheumatoid factors in afflicted joints, together with the presence of large numbers of plasma cells, implicated antibodies as causal agents rather than as secondary consequences of changes in joint pathology induced by other factors.⁷ Not only do autoantibodies serve as biomarkers for different autoimmune disease activity and predict an autoimmune state, but the effects of autoantibodies on target organs are variable. Several findings suggest that there is a relationship between binding and penetration of autoantibodies in the different cell types^{8,9} and the initiation of events leading to various functional cellular alterations.^{10–12} Autoantibodies can thereby modify cell functions, arrest

progression of the cell cycle¹³ and abrogate the expression of some genes.^{14,15}

Autoantibodies penetration into living cells

The capacity of autoantibodies to penetrate cells and induce functional perturbations has been debated for more than 20 years.^{16–27} Although intranuclear autoantibodies deposits have been found in multiple organs of 5%–30% of lupus patients,^{28–30} some have argued that the autoantibodies moved into the cell with tissue fixation, whereas others have suggested that the effects of intracellular autoantibodies are inconsequential, because they are often detected in noninflammatory tissues.³¹ Furthermore, because intracellular transit of large proteins was poorly understood, it was uncertain how large extracellular proteins (eg IgG) transit across the cell membrane, through the cytoplasm and into the nucleus. Not until the first definitive description that a human IgG autoantibody to nuclear RNP could enter into viable human lymphocytes and react with its antigen within the nucleus,¹⁹ a long-standing dogma held that antibodies could react with their respective antigens exclusively in the extracellular compartment. This idea seemed abstruse by itself, for it limited the setting of the humoral immune response to less than one-third of the total body water space. Although autoantibodies directed to intracellular antigens, found in the serum of patients with different autoimmune diseases, were considered as important and useful diagnostic tools, they were never attributed a direct pathogenetic role other than their participation in immune complex-mediated injury. Should immune complex disease be the only potential immunopathogenic mechanism for autoantibodies, one would expect all autoimmune diseases to be nonorgan-specific and bear a very large clinical spectrum. Following the initial demonstration that autoantibodies could enter into cells, a growing number of papers dealing with the penetration of many other antibodies into a large number of animal and human cells have confirmed the phenomenon and provided evidence that the interactions of autoantibodies with intracellular antigens may affect intracellular functions which, in turn, might explain physiopathological and clinical features of autoimmune diseases.³²

Effects of antibody penetration

The formerly prevalent concept that intact autoantibodies could not penetrate into viable cells has been defeated by a large amount of experimental findings and clinical observations that indicate otherwise.

Alarcon-Segovia and colleagues showed for the first time that antibodies of the IgG class against RNA–protein complexes such as the spliceosomal U snRNPs U1 can penetrate into subsets of human T lymphocytes, induce an arrest of the cycle in the G0/G1 phases, and ultimately trigger active cell death.^{32,33} Antibodies to dsDNA that penetrate into human lymphocytes induce both an abnormal activation pathway, as determined by the expression of several activation antigens, as well as apoptosis of a large fraction of penetrated cells.^{15,34} Antibodies directed to dsDNA are also capable of causing podocyte fusion after penetrating glomerular renal cells,³⁵ and antiribosomal P-protein antibodies result in decreased synthesis of apolipoprotein B and cholesterol accumulation after penetrating hepatocytes.³⁶ Penetration of autoantibodies into neural cells has been documented in several instances. Antibodies to the neuronal antigen Hu, present in the sera of some patients with small cell lung cancer, have been shown to penetrate in central nervous system cells and possibly participate in the pathophysiology of the paraneoplastic neuropathy of such patients;³² antibodies to recoverin, a 23 kDa retinal protein, can penetrate photoreceptor and bipolar cells of the retina and induce apoptosis which, in turn, might explain the retinal cell damage and visual loss, without evidence of local inflammatory phenomena, observed in patients with these antibodies;³⁷ antibodies to dsDNA have also been shown to penetrate into rat primary cortical neurons, either alone or serving as carriers for other proteins.³⁸ Antibodies to heat shock protein 27 (hsp27), which are found in patients with glaucoma, have been shown to penetrate into human retinal neuronal cells and induce their active death, most likely by inactivating the ability of hsp27 to stabilize actin cytoskeleton,³⁹ thus suggesting a pathogenetic role of these antibodies. Patients with demyelinating IgM monoclonal neuropathy bear serum antibodies to myelin-associated-glycoprotein and to sulfated glucuronosyl glycolipids, which are capable of penetrating into the myelinated fibers and endoneurial space.^{40,41} Furthermore, it has been suggested that autoantibodies to several nervous system intracellular antigens, elicited by cell damage caused by environmental chemicals, may play a key role in the progression of neurodegenerative diseases.⁴²

Several findings support the fact that antibody penetration takes place *in vivo* as well. These include, among others: the finding of intranuclear immunoglobulins in viable epidermal cells and lymphocytes from patients with mixed connective tissue disease with high titers of serum antibodies to RNP;³² proteinuria following penetration of glomerular cells by anti-dsDNA antibodies in rodents;³² localization of anti-Ro/SSA

antibodies in heart cells of neonates with complete heart block born to women with high serum titers of such antibodies;³² diminution of the cytosolic-mitochondrial phosphorylation potential of adenosine triphosphate (ATP) in myocardial cells after penetration of antibodies to the adenosine diphosphate (ADP)/ATP carrier;⁴³ localization of antinucleolar antibodies in nucleoli of kidney and liver cells in the mercury-induced systemic disease of mice of the histocompatibility (H-2s) genotype.⁴⁴ Perhaps the most interesting effect of antibody penetration is the induction of active cell death.

Regarding the underlying mechanisms, it has been proved that human lymphocytes express exuberant amounts of CD95/Fas after penetration of anti-dsDNA antibodies and mitogen-driven activation,^{32,34} strongly suggesting that the Fas/Fas ligand interaction is involved in activated lymphocytes; however, when resting lymphocytes are exposed to exactly the same anti-dsDNA antibodies, the cells undergo apoptosis without expression of cell surface CD95.⁴⁵ Antibodies to hsp27 have been shown to induce apoptosis of penetrated cells apparently through the interference of such antibodies with the capability of hsp27 to stabilize the cytoskeleton structure,³⁹ and intracellular antibodies to cysteine aspartate protease-3 (caspase-3), the executioner of programmed cell death, have been shown to induce self-activation of caspase-3 moieties, which results in irreversible cell death.⁴⁶ These findings are consonant with the idea that once inside the cells, autoantibodies could directly trigger other pro-apoptotic pathways depending on their antigen specificity.³²

The physiological or pathogenetic role of apoptosis induced by penetration of autoantibodies also appears to be manifold. As mentioned above, active death of cells of the nervous system caused by penetration of antibodies to the Hu antigen, recoverin, hsp27, myelin-associated glycoprotein, sulfoglucuronyl glycolipids, and others, contributes to the immunopathogenesis and clinical features of the diseases where these antibodies are present.^{32,37,39} Similarly, antibodies to the Ro/SSa antigen and to the ADP/ATP carrier that penetrate into heart cells participate as late effectors of the pathophysiology of heart block and myocarditis, respectively.⁴⁷

Recently, our reports extend these observations and shed light on the poorly understood aspect of the apoptotic mechanisms observed in SS, a chronic, incurable autoimmune exocrine disease that is more frequent in women than in men. We demonstrated that anti-Ro and anti-La autoantibodies, strongly associated with SS and present in 70%–90% of patients, bind and penetrate the salivary gland cells, and cause

cellular dysfunction through activation of both the intrinsic and extrinsic pathways of apoptosis.^{48–55}

The penetration of autoantibodies into living cells seems to participate in the pathogenesis of diverse autoimmune diseases, but it may also play a physiological role in healthy individuals. Although the fine mechanisms of the phenomenon remain to be elucidated, the potential use of penetrating autoantibodies as vectors to deliver molecules into cells, with diverse therapeutic purposes, has gained growing interest during the last few years.

Molecular basis of antibody penetration

The mechanisms by which antibodies transgress the cell membrane, travel through the cytoplasm and reach their antigens, either in the nucleus or in other organelles, are still very obscure. Many of the proposed pathways involve the expression of the specific or a cross-reactive antigen at the cell surface level, while others implicate receptors for the Fc fragments of the antibody molecules.^{19,56–58} Concerning the trans-cytoplasmic transit of antibodies, a large variety of mechanisms have been proposed and, although they are not mutually exclusive, no general agreement has been reached. While some investigators have reported that antibodies are internalized in clathrin-associated vesicles and later released by pH or hypotonic lysis of the pinosome,^{59,60} others have not confirmed the participation of pinocytotic vesicles in the phenomenon, but instead have proposed different mechanisms of free transit of antibodies through the cytoplasmic space with the aid of other molecules, such as myosin I protein acting as a chaperone or anti-Ro/La autoantibodies as facilitators of nuclear import of other autoantibodies.^{56,61,62} Recently, it was suggested that some carriers of the ABC pump family, or similar molecular carriers, might be involved in antibody internalization and transport, since several calcium-channel blockers provoke intracellular accumulation of anti-dsDNA antibodies.⁶³

Considering that the autoantibodies target intracellularly located antigens, recent findings supposed that, under certain conditions, receptors for the Fc fragment of IgG (FcγRs) are involved in the penetration of antibodies into cells and FcγRs constitute one of the main effector mechanisms through which autoantibodies exert their action. For example, studies with antinuclear antibodies of patients with SLE, an autoimmune disease with skin involvement,⁶⁴ have suggested that autoantibodies might enter the cells via FcγRs.¹¹ Considering that these autoantibodies are also targeting intracellularly located antigens in keratinocytes, one could imagine that under

certain conditions the FcγRs are involved in the penetration of antibodies into cells.⁶⁵

Fc gamma receptors

Receptors for the Fc fragment of antibodies (Ab) represent the link between the humoral and cellular immune responses. There are several different types of Ab, so called isotypes. The isotype of an Ab is dependent on what Fc part that is expressed in the heavy chain. In humans, there are five different Ab isotypes; IgM, IgD, IgG, IgE, and IgA. IgD, IgE, and IgG are expressed as monomers while IgA and IgM can be expressed as dimers and pentamers, respectively. IgG and IgM are the only isotypes that can activate complement. IgA is the most abundant Ab isotype in the body, present in the respiratory and gastrointestinal tracts, while IgG (Figure 1a) is the most frequent Ab isotype found in the blood.⁶⁶ Further, IgG exists in four subclasses; IgG1, IgG2, IgG3, and IgG4. The specific IgG subclass produced during an immune, or autoimmune, response depends on the type of pathogen or antigen to which the immune system is reacting.⁶⁷

The different Fc parts of IgG subclasses engage various FcγRs on specific leukocytes with varying binding affinities.

In humans, three different types of FcγRs (Figure 1b) belonging to the Ig gene superfamily have been identified: FcγRI (cluster of differentiation (CD64), FcγRII (CD32),

and FcγRIII (CD16), of which the eight genes are clustered on the long arm of chromosome 1 (1q21–23). Extensive structural diversity among FcγRs family members leads to differences in binding capacity, distinct signal transduction pathways, and cell type-specific expression patterns.^{68–71} Such diversity allows IgG complexes to activate a broad program of cell functions relevant to autoimmunity, inflammation, and host defense against microbes and cancer.

FcγRI binds with high affinity human IgG1 and IgG3 whereas FcγRII and FcγRIII bind (with low affinity) IgG under the form of complexes. Human low affinity FcγRs react with all human subclasses with preference for IgG1 and IgG3^{72,73} (Table 1). FcγRI are expressed on macrophages, whereas FcγRII and FcγRIII have a widespread distribution; FcγRII are present on B lymphocytes, macrophages, polymorphonuclear cells, platelets, and monocytes, and FcγRIII are found on granulocytes, natural killer (NK) lymphocytes, macrophages, and activated monocytes (Table 1).

The FcγRs represent type I transmembrane proteins (Figure 1b). Their ectodomains consist of either two (FcγRII, CD32; FcγRIII, CD16; or three (FcγRI, CD64) related Ig domains, whereby the higher affinity of the FcγRI is attributed to the third extra domain. FcγRII has a wide distribution on immunocompetent cells and occurs in two forms, FcγRIIa and FcγRIIb, their extracellular regions sharing 93% sequence identity. These two forms of the receptor can be distinguished by their binding characteristics to IgG subclasses. The transmembrane form of FcγRIII (FcγRIIIa) is present on T cells and NK cells, while a glycosyl phosphatidyl inositol (GPI) anchored form is expressed in high numbers on neutrophils (FcγRIIIb).⁷⁴

FcγRs contain a presumably helical transmembrane region and a cytoplasmic tail which mediates the signal into the cell after the receptors are crosslinked through binding of immune complexes. The cytoplasmic regions contain either an immunoreceptor tyrosine-based activation motif (ITAM) as in FcγRIIa or the respective inhibitory motif (ITIM) as in FcγRIIb. FcγRIIa, FcγRIIIa, and FcγRI are associated with ITAM-containing gamma chains which perform the signaling activity of these receptors. Both of these motifs interact with SH2 domain-containing proteins to initiate signal transduction.^{75–77} The g-chain is a homodimeric, small type I transmembrane proteins that carry a short extracellular part consisting of only five amino acid residues, including the cysteine that mediates homodimer formation. The transmembrane part of this molecule interacts via an Asp/Arg pair⁷⁸ with the receptor while the intracellular part is capable

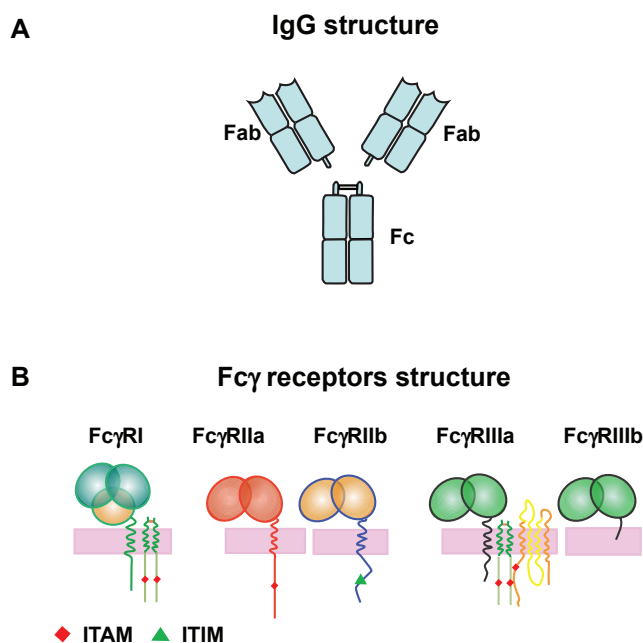


Figure 1 A) schematic structure of IgG molecule. B) schematic representation of the human FcγRs family members.

Abbreviations: FcγR, receptors for the Fc fragment of IgG; IgG, immunoglobulin G.

Table 1 Human FcγRs family members: distribution on immune system cells, affinity IgG-binding, chromosomal localization and function

Name	FcγRI (CD64)	FcγRIIIa (CD32)	FcγRIIb (CD32)	FcγRIIIa (CD16)	FcγRIIIb (CD16)	FcR neonatal
Tissue distribution	Macrophages Dendritic cells	PMN Macrophages Dendritic cells Mast cells	PMN Macrophages B cells Mast cells	NK cells Macrophages Platelets Mast cells PMN	PMN Macrophages B cells Mast cells T cells	Intestinal epithelium Monocytes Intestinal macrophages Dendritic cells
Details	High affinity receptor Binds IgG1 and IgG3 Chromosome 1q21	Low affinity receptor Binds IgG2 Chromosome 1q23	Low affinity receptor Binds IgG2 Chromosome 1q23	Medium affinity receptor Binds IgG1 and IgG3 Chromosome 1q23	Medium affinity receptor Binds IgG1 and IgG3 Chromosome 1q23	High affinity receptor Binds maternal IgG Chromosome 19q13.3
Function	Phagocytosis Cytokine stimulation Dendritic cells- endocytic transport	Phagocytosis Cytokine stimulation Dendritic cells- endocytic transport	Phagocytosis Cytokine stimulation Dendritic cells- transport	Phagocytosis Cytokine stimulation Dendritic cells- endocytic transport	Inhibits ITAM mediated responses. This is the inhibitory receptor	Receptor responsible for the transport of maternal IgG across the cells

Abbreviations: FcγR, receptors for the Fc fragment of IgG; IgG, immunoglobulin G.

of signal transduction. FcγRIIIb lacks a transmembrane and a cytoplasmic region and an immune response cannot be triggered directly by this receptor. It is still not clear how FcγRIIIb transduces signals into the cell as no direct contact to the cytoplasm exists. The FcγRs represent a group of homologue receptors with sequence identities in the range of 50%⁷⁹ (Figure 1b).

Via the binding to FcγR-positive cells, immunocomplexes trigger several functions such as endocytosis, antibody-dependent cell-mediated cytotoxicity (ADCC) and the release of mediators, making them a valuable target for the modulation of the immune system. Fc receptors are, than, molecules that enable antibodies to perform several biological functions by forming a link between specific antigen recognition and effector cells⁸⁰ (Table 1).

How Fcγ receptors bind IgG

The Fc region is separated from the antigen binding parts of the IgG molecule by a flexible hinge region and forms two structural domains, the CH2 and CH3 domains. Cellular and structural approaches have shown that the lower hinge region contains the major binding site for FcγRs. It is established that cross-linking of FcγRs membrane molecules is a prerequisite to IgG-mediated cell activation. Since the Fc portion is composed of two identical polypeptide chains which are related to each other by a two-fold axis, each IgG molecule may potentially bind two FcγRs and initiate cellular responses even in the absence of multivalent antigen.

However, equilibrium sedimentation experiments performed with soluble FcγRII and FcγRIII have shown that the stoichiometry of the interaction of low affinity FcγR with IgG is 1:1, in solution. Studies by nuclear magnetic resonance spectroscopy provided an explanation to this paradox, suggesting that a rearrangement occurs in the lower hinge of one heavy chain upon binding of one FcγR molecule. This small conformational change may preclude the binding of a second FcγR to the second heavy chain Fc.⁸¹ The Fc-FcγRIII co-crystal structures confirmed the 1:1 stoichiometry and showed that the horse shoe-shaped Fc is slightly more opened at the N-terminus of the CH2 domains in the FcγRIII-Fc complex compared with other unligated Fc structures.⁸² The crystal structures of the extracellular domains of FcγRII⁷⁶⁻⁸³ and FcγRIII⁸⁴ show remarkable similarity. The receptors consist of two extracellular Ig-like domains, D1 and D2, with acute interdomain hinge angles of 50–55°, unique to Fcγ receptors, and with Fc-binding region located in the D2 domain. The recent crystal structure of the FcγRIIIb-Fc fragment of IgG1 complex has revealed that the receptor

binds asymmetrically to the lower hinge region of both Fc heavy chains, creating a 1:1 receptor ligand stoichiometry.^{82,85} Low affinity Fc γ R have a low affinity for monomeric IgG. Their biological role is indeed to bind immune complexes. Parallel Fc γ RIIIb dimers have been observed in the crystal lattice. Such dimerization may occur on the cell surface, increasing the avidity of the interaction and subsequently facilitating cell activation.^{84,86}

Expression of Fc γ receptors on nonmyeloid cells

The expression of Fc γ receptors on the surface of nonmyeloid cell types has not been closely studied. Investigation of Fc γ receptors distribution has been carried out to identify the Fc γ Rs members on human epidermal keratinocytes,⁸⁷ human and murine astrocytes,⁸⁸ rabbit liver cells,⁸⁹ human sensory neurons,⁹⁰ human endothelial cells,⁹¹ and human fibroblasts.⁹² Recently our laboratory demonstrated the expression of Fc γ RI, Fc γ RII, and Fc γ RIII on human salivary gland epithelial cells^{49,51} (Figure 2).

Fc γ receptors functions

Fc γ Rs bind IgG and can initiate various functions that can be classified into three major categories.

First, the most prominent function of Fc γ Rs, established by numerous studies over the past several years, is the positive

and negative regulation of cellular responses. Engagement of Fc γ Rs triggers a plethora of biological functions such as phagocytosis, cytolysis, degranulation, and the transcriptional activation of cytokine genes, leading to inflammatory cascades.⁸⁰

The second function is the uptake of immunocomplexes (ICs). Fc γ Rs can internalize the captured ICs leading to homeostatic degradation of the complexes as well as directing the degraded antigenic peptides to the antigen presentation pathway. Macrophages take up and degrade ICs efficiently, whereas dendritic cells are more specialized for antigen presentation. The degradation is, of course, important for elimination of the antigen, a central purpose of the immune system.⁸⁰

The third function is the IgG transport. Fc γ Rs can transfer antibodies transcellularly. The major histocompatibility complex (MHC) class I-related neonatal Fc receptor plays a central role in delivering IgG within and across cells. Neonatal Fc receptor was originally identified as a distant member of the MHC class I protein family. Neonatal Fc receptor is highly expressed in the neonatal rodent gut, where it mediates the uptake of IgG from milk, and in adult tissues such as the vascular endothelium, where it is thought to perform its IgG protection function. Using the gene-deficient mice, neonatal Fc receptor was shown to play pivotal roles in perinatal IgG transport and protection of IgG from

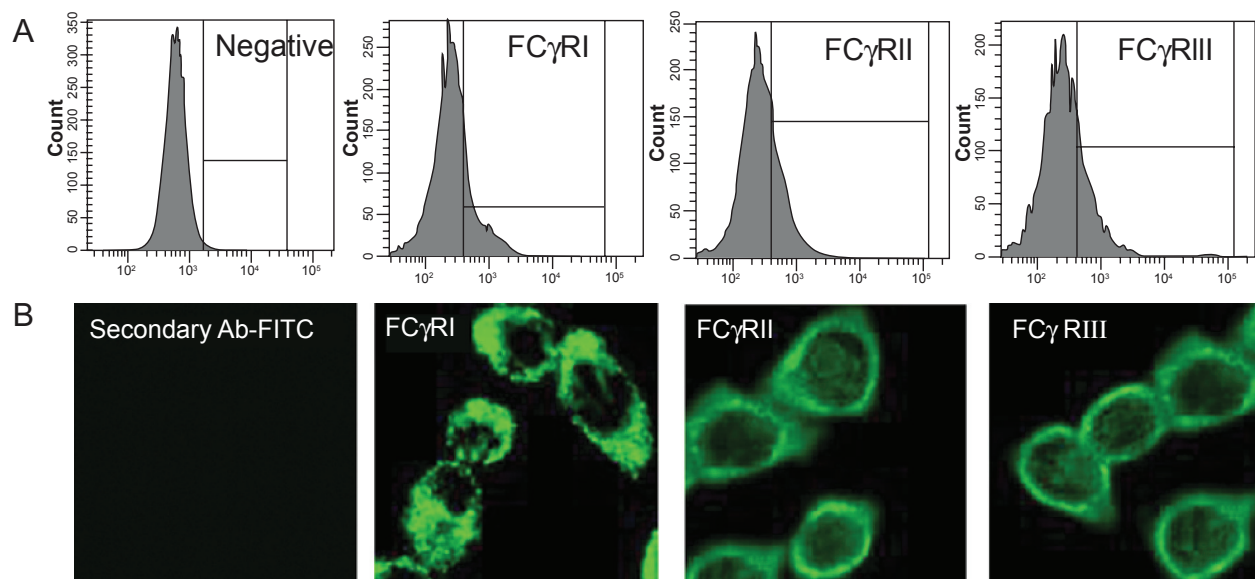


Figure 2 Fc γ Rs family members expression in human salivary gland epithelial cells. **A)** flow cytometric analysis of Fc γ RI, Fc γ RII and Fc γ RIII receptors expression on human salivary gland epithelial cell membrane. Example of flow cytometric images from one representative experiment. Fc γ RI, Fc γ RII, and Fc γ RIII receptors expression was assessed with mAbs mouse anti-human Fc γ RI biotin, mouse anti-human Fc γ RII biotin and mouse anti-human Fc γ RIII biotin. Streptavidin-RPE was used for secondary detection. **B)** confocal microscopy of Fc γ RI, Fc γ RII and Fc γ RIII receptors expression in human salivary gland epithelial cells. Cells were treated with biotinylated anti-human Fc γ RI, biotinylated anti-human Fc γ RII and biotinylated anti-human Fc γ RIII. Streptavidin (FITC) was used for FITC secondary detection.

Abbreviations: Fc γ R, receptors for the Fc fragment of IgG; FITC, fluorescein isocyanate; IgG, immunoglobulin G.

catabolism.^{93,94} In addition to these three major functions of FcγRs, compelling evidence exists that some types of FcγRs are released into blood as a soluble form and these soluble FcγRs modulate immune responses⁹⁵ (Table 1).

Roles of human Fc gamma receptors in autoimmunity

The development of autoimmune diseases is complex and dependent on multiple genes and environmental factors. It is preferable to consider genetically engineered animal models for autoimmune disease before considering FcγRs mechanisms for the development of human autoimmune disease. Mice deficient in the FcR gamma chain or activating type FcγRs are resistant to the induction of, or spontaneous onset of, various autoimmune diseases and hypersensitive reactions.⁹⁶ These results suggest that a wide range of inflammatory and autoimmune diseases, such as vasculitis, glomerulonephritis, and autoimmune hemolytic anemia, may be mediated by FcγRs and not, as previously thought, primarily by complement factors, although in several IC induced inflammatory and hypersensitive reactions a combinatory function of FcγRs and complement activation is demonstrated.^{97–99}

In RA studies have been performed analysing the cellular expression of FcγRs and genetic polymorphisms of FcγRs in relation to RA susceptibility have been investigated. Accordingly, the percentage of FcγRIIIa-positive monocytes in peripheral blood is augmented^{100,101} and the expression level of FcγRI, FcγRII, and FcγRIIIa on RA monocytes is increased compared to healthy individuals.^{102–104} An upregulation of activating FcγRs has also been observed in RA synovial tissue compared with synovia from trauma or osteoarthritis patients.^{105–107} Indirect proof of that FcγRs play a role in the pathogenesis of RA is that several effective RA therapies have modulatory effects on the FcγR expression. For example, infusions of infliximab, an anti-TNF Ab, reduced the expression of FcγRIIIa on neutrophils in RA patients whereas the expression of FcγRIIb was induced.¹⁰⁸ Infliximab treatment also reduced the expression of FcγRI on peripheral blood monocytes, while the expression of FcγRIIIa and FcγRIIIa was unaffected.¹⁰⁹ The reduction of FcγRI was accompanied by a decrease in the levels of the erythrocyte sedimentation rate and C-reactive protein (CRP), which are indicative of inflammation. Moreover, methotrexate, a cytostatic widely used to treat aggressive RA, decreased the FcγRI and FcγRIIIa expression on circulating monocytes.¹¹⁰ The decrease in FcγRI expression was correlated with a decline in CRP levels and increase in the wellbeing of the patients.

Administration of glucocorticoids also has effects on FcγRs and reduces the expression of FcγRI and FcγRIIIa on blood monocytes and decreases the amount of FcγRIIIa positive cells.^{102,103,111}

Regulation of human Fcγ receptor expression

Little is known about how the expression of these receptors is regulated in nonmyeloid cells. According to previous reports on IFN-γ upregulation of FcγRIII proteins on neutrophils¹¹² and eosinophils,¹¹³ Cauza and colleagues demonstrated that (interferon-γ) IFN-γ treatment induces significant upregulation of FcγRIII on cultured human keratinocytes,⁶⁵ and an increase was shown in the abundance of FcγRs during *in vitro* activation of rat hepatic stellate cells with IgG.¹¹⁴ FcγRs are strongly upregulated on macrophages in synovial tissue and blood monocytes in RA patients.¹¹⁵

Our recent publications documented that anti-Ro and anti-La autoantibodies characterizing SS determine an increase of FcγRs expression on human salivary gland epithelial cells.^{49,51} We provided evidence of upregulation of both the high affinity FcγRI and the low affinity FcγRII and FcγRIII. This finding strengthens the idea of a direct involvement of FcγRs in the pathogenic role of anti-Ro and anti-La autoantibodies in SS. FcγRs not only mediate the uptake and transport of autoantibodies in salivary gland cells, but could contribute in various ways to the onset and/or progression of autoimmune diseases^{49,51} (Figure 3).

Emerging therapeutic potential for Fcγ receptors

The structural heterogeneity and complex nature of FcγR isoforms and their variant alleles reflect the diverse functions mediated by these receptors. Recognition of the role of FcγRs in the pathogenesis of immune-mediated disease suggests multiple avenues for potential therapeutic intervention. There has been a renewed interest since few years in the use of mAbs in the diagnostic and treatment of various autoimmune diseases.^{116–118} The most impressive clinical results have been obtained with a wide array of biological agents designed to inhibit tumor necrosis factor-α (TNF-α), and soluble receptors that bind and neutralize TNF have been developed for the treatment of inflammatory and autoimmune diseases.^{116–118} These new biological treatment modalities include etanercept, a dimeric fusion protein consisting of soluble TNFR75 fused to the Fc portion of human IgG that, by preventing interactions between TNF and its receptor,

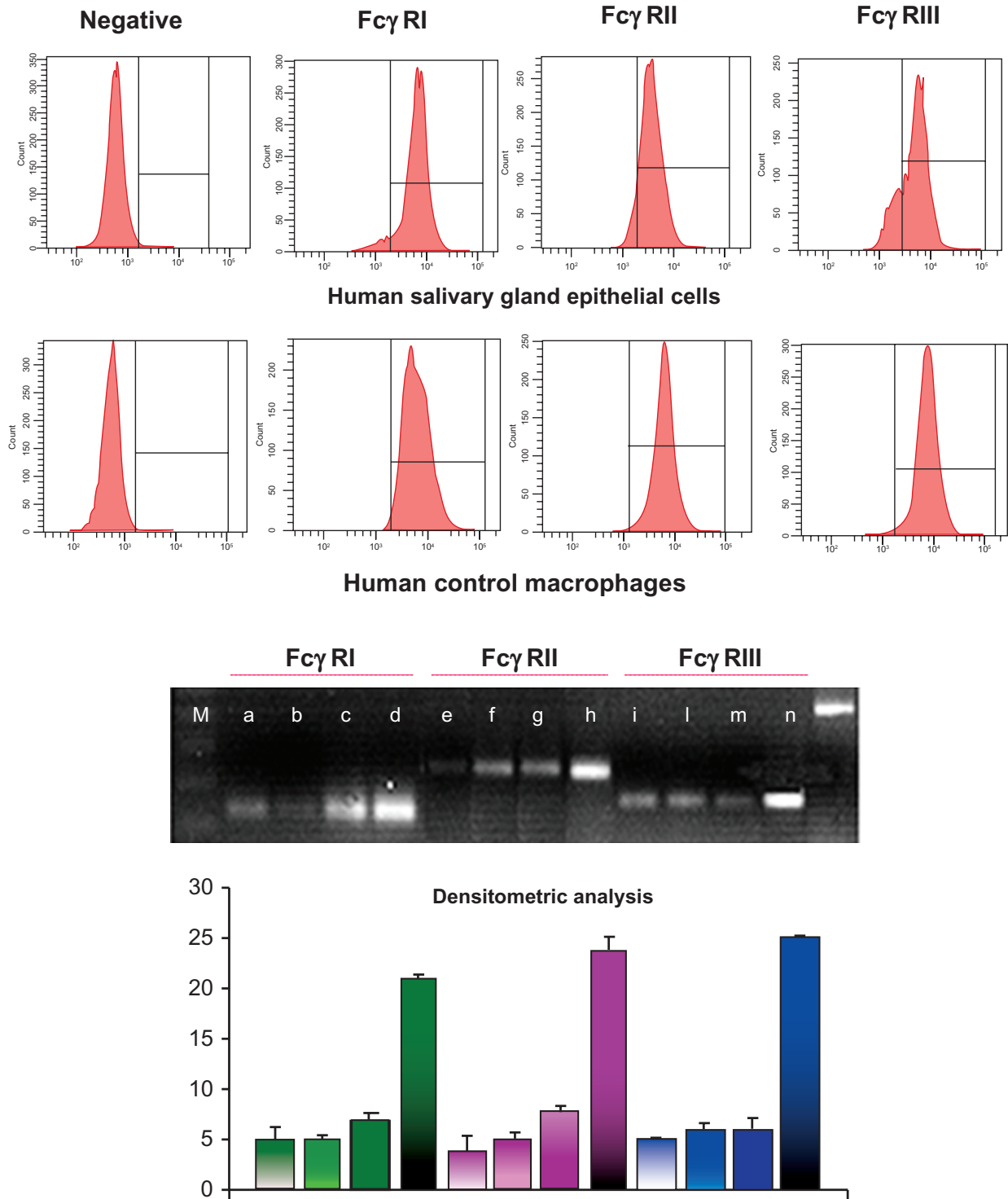


Figure 3 FcγRs expression is increased upon *in vitro* activation of salivary gland cells with anti-Ro and anti-La autoantibodies. Flow cytometric analysis demonstrates the upregulation of FcγRs in human salivary gland epithelial cells, confirmed by agarose gel picture of RT-PCR results and densitometric analysis (**A–D**, analysis of FcγRI expression in cells treated with growing concentration of anti-Ro; **E–H**, analysis of FcγRII expression in cells treated with growing concentration of anti-Ro; **I–N**, analysis of FcγRIII expression in cells treated with growing concentration of anti-Ro).

Abbreviations: FcγRs, receptors for the Fc fragment of immunoglobulin G; RT-PCR, reverse transcriptase–polymerase chain reaction.

neutralizes TNF activity, and adalimumab, a fully human anti-TNF- α human sequences. Adalimumab was developed using phage display technology, a method that mimics natural immunoglobulin gene rearrangement and contains neither nonhuman components nor artificially fused human peptide sequences. It has a high specificity and affinity but not other cytokines, such as TNF- β .^{119–121} There is increasing evidence that the Fc portion of the anti-TNF- α mAbs is a major component of their therapeutic activity, through binding to Fc γ Rs expressed by effector cells present in the autoimmune microenvironment.¹²²

Anti-TNF- α mAb treatment of RA patients is accompanied by downregulation of Fc γ RI expression levels on monocytes. This is likely an indirect effect of TNF- α blockade on disease activity, since *in vitro* anti-TNF- α mAb does not directly change Fc γ RI expression on monocytes. In contrast, TNF- α downregulated all activating Fc γ Rs. Thus, blocking TNF- α may relieve the negative feedback mechanism of TNF- α as downregulator of Fc γ Rs. Strategies to reduce activating Fc γ Rs may have additional value in the treatment of RA patients with TNF- α blockade by diminishing immune complex-mediated activation of monocytes/macrophages.¹²³

Immune activation and inhibitory receptors play an important role in the maintenance of an adequate activation threshold of various cells in our immune system. Analyses of murine models show that the inhibitory Fc γ Rs, Fc γ RIIB plays an indispensable role in the suppression of antibody-mediated allergy and autoimmunity. In contrast, all Fc γ Rs, except for the inhibitory Fc γ RIIB, are essential for the development of these diseases, suggesting that regulation of inhibitory or activating Fc γ Rs is an ideal target as a therapeutic agent.¹²⁴

With advances in structural biology and the recent solution of the three dimensional structure of Fc γ Rs, the design of small chemical entities to inhibit receptor function is now a possibility. The interaction of immune complexes with the human Fc γ Rs initiate the release of inflammatory mediators and is implicated in the pathogenesis of human autoimmune diseases, including RA and SLE. Therefore Fc γ Rs are a potential target for therapy. Recently, small molecule inhibitors were designed, that blocked immune complex-induced platelet activation and aggregation and tumor necrosis factor secretion from macrophages in a human cell line and transgenic mouse macrophages.¹²⁵ These observations identify human Fc γ Rs as appropriate targets for future drug design and/or immunotherapy, with the hope of blocking early inflammatory responses, before cytokine release and tissue damage occur. New therapies arising from

both clinical and experimental studies aimed at providing a better understanding of the role of Fc γ Rs in autoimmunity, offer the hope of improving treatment outcomes in a broad range of chronic and debilitating diseases.

Acknowledgements

We are grateful to MVC Pragnell for a critical reading of the manuscript.

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