CHAPTER 7

FC RECEPTORS AND PATHOLOGY

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INTRODUCTION

The considerable amount of data on soluble and membrane FcR functions accumulated during the last decade has opened the way to a detailed exploration of the role of these molecules in rodent and human pathologies. Two major aspects of the role of FcR in pathology have been investigated: on the one hand, many efforts have been devoted to the study of the genetics of FcR expression, as well as the biochemical and functional characteristics of these molecules in pathology. The level of circulating soluble FcR and the presence of anti-FcR antibodies in sera of patients with various diseases have also been evaluated. On the other hand, FcR have been viewed as exquisite target molecules for immuno-intervention, based on their ability to induce cytotoxicity (ADCC), phagocytosis, endocytosis, release of cytokines and inflammatory mediators, enhancement of antigen presentation, and anergy. This chapter presents and discusses the involvement of the three FcγR (FcγRI, CD64, FcγRII, CD32, FcγRIII, CD16) and of the low-affinity FcεR (FεRII, CD23) in pathology, as well as the potential use of these receptors for immuno-intervention.

GENETIC ASPECTS OF FCR EXPRESSION: RELATIONSHIP TO DISEASES

MOUSE FCR

Autoimmunity

The genes encoding the mouse low-affinity FcγRII (*Fcgr2*) and FcγRIII (*Fcgr3*) are located on the distal part of chromosome 1,[1-3] while

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the gene encoding the high-affinity FcγRI (*Fcgr1*) is encoded on a segment of chromosome 3 between D3Nds6 (*IL-2*) and D3Nds9 (*Adh-1*).[4] Genetic studies aimed at defining a genetic linkage and tumor susceptibility genes in autoimmune diabetes and plasmacytomagenesis, respectively, have suggested that FcγR could be involved in the physiopathology of these diseases (Table 7.1).[4,5]

A congenic, non-obese diabetic (NOD) mouse strain, less susceptible to diabetes than NOD mice, was selected that contains a segment of chromosome 3 with three marker loci [D3Nds7 (*Cacy*), D3Nds11 (*Fcgr1*), and D3Nds8 (*Tshb*)]. This 7-cM region shows strong linkage to diabetes and insulitis. Interestingly, phenotypic analyses of NOD mice looking for single, fully penetrant genes permits the demonstration that NOD mice express high levels of FcγRI on monocytes, macrophages, and neutrophils after a Complete Freund's adjuvant challenge (CFA) by comparison with non-diabetic mouse strains such as C57Bl/10SnJ. This phenotype prompted Prins et al[4] to sequence the FcγRI gene of NOD and C57Bl/10SnJ mice. The B10 sequence was identical to the published one, whereas the NOD coding sequence showed several major differences. A stop codon was found at position 337, which results in a cytoplasmic tail lacking 73 amino acid residues (i.e., 75% of the total length). In addition, 24 single-base differences leading to 17 amino acid changes were also observed. This was related to a 73% reduction in the turn-over of FcγRI-IgG containing complexes present on NOD macrophages. Linkage of mutated FcγRI to insulitis and diabetes is due to increased homozygoty: one dose of the wild-type allele in heterozygous mice is sufficient to provide protection from diabetes. The role of this specific defect on NOD macrophages is not elucidated so far. One hypothesis advanced by Prins et al[4] is increased binding of NOD FcγRI to immune complexes or to monomeric IgG2a, followed by a strong ADCC and/or by the release of cytokines such as TNF-α, that is detrimental to β cells in vitro. It should be noted that amongst the 22 strains tested, only one other was found to carry the *Fcgr1* nonsense mutation, the high antibody responder Biozzi strain (AB/H).[4] A possible underlying mechanism accounting for a role of FcγRI in autoimmune diabetes could therefore be the potentiation of immune responses through the targeting of NOD macrophage FcγRI by IgG-containing immune complexes directed to β cells. Enhancement of antibody response has recently been reported when an anti-human FcγRI antibody containing known antigenic determinant was injected into human FcγRI transgenic mice.[6] However, the involvement of FcγR in autoimmune diabetes may not be restricted only to FcγRI. Recent data obtained by Luan et al in collaboration with our laboratory (manuscript submitted) indicate that a quantitative trait locus for increased IgG1 and IgG2b serum levels in the NOD mouse strain is mapped into the distal chromosome 1 region, close to the *Fcgr2* locus encoding FcγRII. The expression of FcγRIIb2 (membrane-bound)

Table 7.1. Genetic aspects of mouse FcγR expression: relationship to diseases

FcR	Locus	Mouse strain	FcγR Phenotype	Immune Phenotype
FcγRI	*Fcgr 1* (chr 3)	NOD	shorter IC tail	linkage to autoimmune diabetes
"	"	Biozzi high responders	shorter IC tail	enhancement of antibody response
FcγRII	*Fcgr 2* (chr 1)	NOD	weak FcγRIIb2/b3 expression	elevated IgG1 and IgG2 serum levels
FcγRII	*Fcgr 2* (chr 1)	DBA/2N	wild-type	linkage to a tumor susceptibility gene (plasmacytomagenesis)

and FcγRIIb3 (soluble) isoforms of this receptor is strongly decreased on NOD macrophages, leading to a poor binding of IgG1 and IgG2b, which parallels an elevated serum level of these two subclasses. Overall, these observations indicate that the impairment of FcγR expression and functions observed could have important consequences in the regulation of immune responses in NOD mice, with possible implications in the physiopathology of autoimmune diabetes (Table 7.1). Recent studies performed on FcγRII-deficient mice showed that elevated immunoglobulin levels in response to both thymus-dependent and thymus-independent antigens are observed in these animals.[7] In addition, mast cells from these FcγRII$^{-/-}$ mice were found to be highly sensitive to IgG-triggered degranulation, confirming the down regulatory effect of FcγRII on other immune receptors.[8,9]

Plasmacytomagenesis

Fcgr2 has also been linked to a tumor susceptibility gene (Table 7.1).[5] The genetic mapping of tumor susceptibility genes involved in mouse plasmacytomagenesis helped to identify a 32-cM stretch of mouse chromosome 4 near *Gt10* (gene trap insertion 10) (>95% probability of linkage) as well as a susceptibility gene on chromosome 1, likely linked to *fcgr2* (90% probability of lineage). χ^2 and lod score analyses of susceptible and resistant backcross progeny between BALB/c and DBA/2N mice for 10 markers spanning chromosome 1 showed a potential linkage of this susceptibility gene to *Fcgr2*. It has been suggested that the DBA/2N allele of this gene is associated with susceptibility and that

the BALB/c allele is associated with resistance.[5] Interestingly enough, the mouse chromosome 1 shares extensive linkage homology with stretches of the human chromosome 1 previously associated with cytogenetic abnormalities in multiple myeloma. This has led to the proposition that a tumor suppressor gene may reside on human chromosome 1 close to *Fcgr2*.[5,10] A direct involvement of low-affinity FcγR in the physiopathology of mouse plasmacytomas has not been demonstrated so far. However, two sets of data indicate that a careful examination of FcγR expression and functions should still be performed in this pathology: first, it has been shown that the expression of low-affinity FcγR on $CD2^+$ cells (NK and T cells) is increased in myeloma-bearing mice or in patients with IgG-producing multiple myeloma (MMγ).[11] In addition, a stage-related decrease of circulating soluble FcγRIII (sCD16), which lies as its murine counterpart close to the locus encoding FcγRII on human chromosome 1, has been reported in multiple myeloma patients (see below).[12,13]

Human FcR

In man, all three FcγR are localized on chromosome 1 (FcγRI, CD64: 1q21; FcγRII, CD32: 1q23-24; FcγRIII, CD16: 1q23-24).[14,15] In addition, the human T cell receptor (TcR) ζ/η gene which encodes the ζ molecule which is also associated to NK cell FcγRIII,[16] has been linked to the FcγRII-FcγRIII gene cluster on chromosome 1q.[17] Also, both the α and γ chains of FcεRI (the γ chain being also associated to human FcγRI and FcγRIII as homo- or heterodimers with the ζ chain) have been mapped to chromosome 1q22-23.[18]

FcγRI (CD64)

Studies of familial FcγR-related defects as well as of the impact of allelic forms of FcγR on humoral and cellular immunity have provided some insights into the role of FcγR in pathology (Table 7.2). Four individuals have been identified within a single family who fail to express detectable levels of FcγRI (CD64) on their monocytes and macrophages.[19,20] Although their monocytes are unable to support mouse IgG2a anti-CD3-induced T cell proliferation, these individuals are apparently healthy. Thus, failure to express FcγRI does not provoke an entire blockade of phagocytosis in these individuals and has no significant detectable consequences on their clinical condition, especially with regard to infectious diseases. By contrast, it is interesting to point out that targeted disruption of the mouse FcγR-associated γ chain, that affects both FcγRI and FcγRIII expression on macrophages and NK cells, results in immunocompromised animals with an absence of phagocytic activity and a severe impairment of ADCC activity.[21] Thus, the presence of several FcγR with overlapping functional activities probably prevents major dysfunction of immunity in these FcγRI-defective individuals. Molecular analyses showed that all three

FcγRI genes are present, but that only one of the two mRNA species observed in normal donors (1.7 and 1.6 kb) can be detected in each of the four individuals studied (the 1.6 kb species). In addition, a single nucleotide difference within the extracellular domain exon 1-encoding region of FcγRIA has been evidenced, resulting into a termination codon (codon 92).

FcγRII (CD32)

Allelic forms of one of the FcγRII (FcγRIIa) have been described that could have important consequences for the humoral immune response against infectious pathogens (Table 7.2). These allelic variants, first identified on the basis of a functional polymorphism for the binding of mouse IgG1 and of human IgG2, have been termed the high responder (HR) or the low responder (LR) isoforms of FcγRIIa, depending on their ability to activate or not activate T cells via an IgG1 anti-CD3 mouse monoclonal antibody.[22] The HR isoform does not significantly bind human IgG2 by contrast to the LR isoform.[23,24] The molecular basis of this polymorphism has been elucidated.[24] A two-residue difference in the extracellular region has been demonstrated between HR and LR isoforms. The HR isoform contains an arginine at position 131 whereas a histidine is found in the LR isoform. This residue position is critical for the binding of both mouse IgG1 and human IgG2. A change in another position (27), tryptophan being replaced by a glutamine, has been described in both isoforms and does not play any role in this polymorphism. These observations have led to the suggestion that HR subjects could be more prone to recurrent bacterial infections due to their inability to bind human IgG2, the predominant isotype of the humoral response against bacterial capsular polysaccharides. The distribution of LR and HR isoforms, based on differential binding of murine IgG1, is 70% HR and 30% LR in Caucasians, whereas it is reversed in Asians, with 15% HR and 85% LR.[25] PCR-based sequence analysis of genomic DNA has also been used to determine the distribution in healthy individuals.[26] For Caucasian Americans, the distribution is 19% HR/HR, 51% LR/HR and 30% LR/LR, while the distribution was determined to be 26% HR/HR, 60% HR/LR and 14% LR/LR for Africans/Americans. The clinical significance of this polymorphism has been examined in different situations. Forty-eight children with recurrent bacterial respiratory tract infections were analyzed for their FcγRIIa phenotypes.[27] Interestingly enough, the LR/LR phenotype (efficient IgG2 binding) was less than half that observed in a cohort of 123 healthy adults. Conversely, in a retrospective study, 11 of 25 children who survived fulminant meningococcal septic shock were HR/HR (poor IgG2 binding) which is a significantly more frequent rate (44%) than found in healthy Caucasians (23% in this study).[28] In addition, neutrophils expressing the HR phenotype phagocytized *N. meningitidis* opsonized with polyclonal

IgG2 antibodies less effectively than did LR neutrophils in this study.[28] Thus, although a significant correlation between the failure to bind IgG2 through FcγRIIa (HR phenotype) and a higher sensitivity to recurrent bacterial infections could not be strictly demonstrated, these data suggest that FγRIIa could play an important role in host defense against bacterial infections among other factors. For instance, it has been claimed that the virtual complete absence of *Hæmophilus influenzæ* infections in Japan could be due to the predominance of the LR phenotype in Asian populations, allowing the development of IgG2-mediated immune mechanisms, the IgG2 isotype being the main IgG subclass produced following *Hæmophilus influenzæ* infection.[29]

FcγRIII (CD16)

Studies on genetic defects affecting FcγRIII (CD16) expression have shown that some individuals fail to express FcγRIIIb on neutrophils (Table 7.2). In one study,[30] 21 FcγRIIIb negative donors were genotyped for the NA polymorphism of the FcγRIIIB gene (NA-1/NA-2). All the donors were found negative for both the NA-1 and the NA-2 allele. RFLP analysis confirmed the absence of the FcγRIIIB alleles. An additional deletion of the next more telomeric located FcγRIIC gene was also found. Fourteen of the 21 patients never suffered from serious infections, while two had an autoimmune thyroiditis, four from multiple episodes of infection, and three from incidental infections. Thus, as already observed with the high-affinity FcγRI (see above), failure to express one of the two low-affinity FcγR does not provoke marked immunocompromise. It should be noted that in the latter study, the phenotype distribution of FcγRIIa was normal, excluding

Table 7.2. Genetic aspects of human FcγR expression: relationship to diseases

FcR	Locus	FcγR phenotype	Disease
FcγRI (CD64)	1q21 (chr 1)	no expression (gene deletion)	no disease observed
FcγRII (CD32)	1q23-24 (chr 1)	LR/HR allelism (HR: no IgG2 binding)	HR prone to recurrent bacterial infections?
FcγRIII (CD16)	1q23-24 (chr 1)	no expression (gene deletion)	transient neutropenia in newborn infants (allo-immunization)
"	"	NA-1/NA-2 allelism of FcγRIIIb (PMN cells)	lower IgG1/IgG3-mediated NA-2 phagocytosis?

any compensatory phenomenon based on a more frequent LR phenotype in this group of subjects.[30] Failure to express FcγRIIIb on neutrophils, also due to the absence of the FcγRIIIB gene, could be reversed in a patient in first remission of acute myeloid leukemia whose neutrophils were found to lack NA-1 and NA-2 alloantigens: bone marrow transplantation from an HLA-identical sibling allowed to convert this NA null phenotype to the normal phenotype.[31] Also, soluble FcγRIII (sCD16) was absent in the pretransplant plasma of the patient, while 20 units of sFcγRIII were detected 160 days after bone marrow transplantation. Whether this patient subsequently developed antibodies directed against FcγRIIIb has not been documented. However, in five other studies, it has been demonstrated that a moderate neutropenia observed in newborn infants was due to the presence of antibodies to FcγRIIIb, whose expression was undetectable on the mother neutrophils. These antibodies were specifically directed against FcγRIIIb. They did not bind FcγRIIIa, which was normally expressed on the maternal lymphocytes. The neutropenia was transient (reported as a transient neonatal alloimmune neutropenia, NAIN) and rapidly reverted in the children. Strikingly, the absence of FcγRIIIb on the maternal neutrophils, due to FcγRIIIB gene deletion or abnormality, was not associated with any pathology or susceptibility to infections.[32-36] The gene frequency of the NA-null phenotype was calculated as 0.0274 ± 0.0059.[34] No elevated level of circulating immune complexes was observed.[32] One patient with systemic lupus erythematosus (SLE) has also been reported to lack FcγRIIIb expression on neutrophils, but without any evidence for the implication of this defect in the pathology.[37] The absence of FcγRIIIb expression on neutrophils has also been reported in patients with paroxysmal nocturnal hemoglobinuria (PNH).[38] In that case, it is due to an acquired abnormality of hematopoietic cells affecting phosphatidylinositol glycan (PIG) membrane anchoring. By contrast to the observations reported above, this deficiency is associated with high levels of circulating immune complexes and susceptibility to bacterial infections.[38] However, the lack of FcγRIIIb expression could be unrelated to these clinical observations, as this PIG-membrane anchoring deficiency also affects other PI-linked molecules (such as decay accelerating factor or DAF, and LFA-3) that could play an important role in the clinical effects observed in PNH patients.

The role of the NA-1/NA-2 allelism[39] in the efficiency of IgG subclass-mediated phagocytosis of various bacteria and on the IgG-mediated rosette formation and phagocytosis of Rhesus D^+ human red cells has also been compared.[40] The molecular basis of this polymorphism has been elucidated.[41,42] When FcγRIII is deglycosylated, the NA-1 form exhibits a mass of 29 kDa, the NA-2 form a mass of 33 kDa. The polymorphism is due to a four-amino-acid difference between the two forms, which results in the loss of two-linked glycosylation sites in the NA-1 form (four sites vs six sites). The phenotypic frequencies of the

NA-1 and NA-2 forms in Caucasians are 37% and 63%, respectively.[43] Interestingly, IgG1-mediated phagocytosis of *Staphylococcus aureus* strain Wood, *Hæmophilus influenzæ* type b, and *Neisseria meningitidis* group B has been found lower with NA-$2^{+/+}$ neutrophils than with NA-$1^{+/+}$ neutrophils. Similarly, IgG3 anti-D-mediated rosette formation and phagocytosis was also lower with NA-$2^{+/+}$ neutrophils.[40] Although this study shows an influence of the FcγRIII NA-1/NA-2 allotypes in functional interactions with biologically relevant IgG subclass antibodies (Table 7.2), whether it is related to a differential ability to mount immune responses to bacterial pathogens remains to be established.

MEMBRANE-BOUND FcγR: INVOLVEMENT IN AUTOIMMUNITY AND INFLAMMATION

MOUSE FcγR

The interaction of IgG-containing immune complexes with FcγRs expressed on various cell types results in a variety of events, from internalization of immune complexes eventually followed by the antigen processing and presentation[44] to the release of cytokines or inflammatory mediators.[15,45-54] Thus, autoantibodies directed against FcγR could trigger pathogenic events, as has been previously shown for antibodies directed against other surface receptors such as anti-acetylcholine receptor antibodies.[55] High levels of anti-FcγR autoantibodies have been found in mice prone to autoimmune diseases (NZB, NZB/NZW, Tightskin).[56] Monoclonal IgM anti-FcγR antibodies derived from these animals comprise a large subset of polyspecific IgM mAbs (60%). Both serum from these mice and anti-FcγR IgM mAbs were able to inhibit the binding of immune complexes to macrophages.[56] These antibodies could be responsible for the impairment of the macrophage functions observed in these animals.[57] In addition, these anti-FcγR antibodies induce the release of hydrolases from both azurophilic and specific granules of human neutrophils.[58] This release occurs at very low concentrations of IgM mAbs and is likely to be important in inflammation accompanying autoimmunity.

HUMAN FcγR

Similar observations have also been made in humans. For instance, a monoclonal IgG2 anti-FcγRIII antibody which has been derived from a patient with progressive systemic sclerosis, triggers the release from neutrophils of β-glucuronidase, arylsulfatase and alkaline phosphatase.[59] The presence of anti-FcγR antibodies has been reported in patients with juvenile neutropenia (see above), SLE, and localized and systemic scleroderma.[32-37,60,61] A detailed analysis of the serum from 147 patients with different systemic autoimmune diseases (SLE, Sjögren's syndrome, and progressive systemic sclerosis) showed that different patients have autoantibody directed against each of the three human FcγR.[62] Affinity

purification of seven positive sera indicated that these anti-FcγR Ig belonged either to IgM or IgG isotypes, with a yield of 1.5-6 μg/mL of affinity purified protein per mL of serum. Besides activating FcγR$^+$ cells, anti-FcγR antibodies could block efficient clearance of immune complexes by the mononuclear phagocyte system, leading therefore to a higher serum concentration of immune complexes and contributing to immune complex deposition in the kidney. In another study, high titers of circulating IgM reacting with both FcγRII and FcγRIII were found in SLE and rheumatoid arthritis patients. By contrast, sera from patients with Raynaud's syndrome showed predominantly anti-FcγRIII IgG. Patients with progressive systemic sclerosis showed both anti-FcγRII and anti-FcγRIII IgG and IgM. Last, many patients diagnosed with degenerative osteoarthritis also had IgG autoantibodies, directed primarily against FcγRII. No significant incidence of anti-FcγR Ig was observed in healthy individuals in this study.[63] Studies are currently pursued in different laboratories for examining the correlation of anti-FcγR autoantibodies with the clinical course of various autoimmune diseases.

SOLUBLE FcγR AND FcεR IN PATHOLOGY: MARKERS WITH A DIAGNOSIS AND/OR PROGNOSIS VALUE?

Soluble forms of FcR (sFcR) have been described in the early 70s.[64] They were first termed immunoglobulin binding factors (IBF), as they retain the ability to specifically bind IgG like their membrane counterparts.[65] sFcR derived from almost all the different isotype-specific membrane receptors were then described (sFcγR, sFcαR, sFcεR, sFcδR).[66] They are produced either by proteolytic cleavage from their membrane counterparts or by differential splicing of their transmembrane exon.[67]

sFcγR molecules have been found in the supernatant of cells cultured in vitro or in biological fluids such as sera or saliva.[68-75] Studies to define the biological role of sFcγR from mouse or human origin have led to the conclusion that they could act as potent competitors of membrane-associated FcγR by preventing IgG-FcγR interactions that are necessary to trigger effector functions such as antibody-dependent cell cytotoxicity (ADCC) or capture and internalization of immune complexes.[67,75,76] In addition, it has been claimed that sFcγR, as well as sFcεR and sFcαR, are involved in the control of antibody production of both normal and tumor B cells such as hybridoma B cells.[65,67,75,77-81] sFcR have been also implicated in the control of B cell proliferation.[12,82-84] However, this has not been proven yet using recombinant molecules, suggesting that these regulatory effects could be mediated by other molecules having structural and/or functional relationship with sFcγR. Notably, it has recently been shown that TGF-β, also capable of binding the Fc region of IgG, could be responsible for the suppressive effect on B cell proliferation first attributed to sFcγR.[85] Finally, the ability of both membrane-associated and soluble forms of FcR to bind

ligands other than Ig such as CD11b/CD18 (CR3) and CD11c/CD18 (CR4) opens the way to a larger variety of biological effects of these molecules than previously thought.[86-90]

These different observations have brought considerable interest in studying the level of circulating FcR in biological fluids from animals or patients with various pathologies as described below and summarized Tables 7.3 and 7.4.

Table 7.3 Soluble human FcγRIII and diseases

Human FcR	Disease	Serum level
FcγRIII (CD16)	MM[a]	stage-related decrease (low or absent in stage III patients)
"	MM + IVIG (in stage III patients)	increase
"	MGUS[a]	no change
"	ITP[a]+ Fcγ fragments	increase
"	HIV$^+$ patients[a]	drop in AIDS patients
"	B-CLL[a]	no change
"	AML[a]	decrease
"	ARDS[a]	increase (bronchoalveolar lavage fluid)
"	autoimmune-related disorders (SLE, RA, Sjögren)[a]	increase (heterogeneous)
"	ASA[a] (antisperm antibodies)	decrease (seminal plasma)

[a]MM: multiple myeloma; MGUS, monoclonal gammopathies of unknown significance; ITP, idiopathic thrombocytopenic purpura; HIV, human immunodeficiency virus; B-CLL, B chronic lymphocytic leukemia; AML, acute myelogenous leukemia; ARDS, adult respiratory distress syndrome; SLE, systemic lupus erythematosus; RA, rheumatoid arthritis; ASA, antisperm antibody syndrome.

SOLUBLE FCγR AND PATHOLOGY

Mouse soluble FcγR

In mouse, sFcγR levels are upregulated upon antigenic stimulation, allogeneic stimulation or infections with parasites (*Trypanosoma cruzi*) or viruses such as LDV (lactate dehydrogenase virus).[91,92] It is likely to reflect the stimulation of immune cells upon antigenic challenge, which leads to the release of sFcγR. This increase could be due

Table 7.4 Human soluble FcγRII and FcεRII and diseases

Human FcR	Disease	Serum level
FcγRII (CD32)	MM[a]	no change (moderate increase in stage I patients?)
"	ITP[a]+ Fcγ fragments	no change
"	B-CLL[a]	increase in stage C patients (Binet staging)
"	SLE[a]	moderate partial increase[b]
"	Behçet 's disease	moderate partial decrease[b]
FcεRII (CD23)	B-CLL	stage-related increase (elevated in stage II and III, Rai staging)
"	HIV+ patients	increase before AIDS-associated NHL[a] appearance
"	autoimmune-related disorders (SLE, RA, Sjögren)[a]	increase
"	autoimmune-related disorders (Coeliac and Crohn's diseases)[a]	decrease

[a] MM: multiple myeloma; ITP, idiopathic thrombocytopenic purpura; HIV, human immunodeficiency virus; B-CLL, B chronic lymphocytic leukemia; SLE, systemic lupus erythematosus; RA, rheumatoid arthritis; NHL, non-Hodgkin's lymphoma.

[b]only a patient subgroup appears to exhibit a significant change in FcγRII serum levels, without any correlation with other biological or clinical parameters.

to the production of cytokines that affect FcγR expression. For instance, it has been shown that IFN-γ, a cytokine that is induced during parasitic or viral infections, upregulates both the membrane expression of FcγR[93,94] and the release of sFcγR.[95] Similarly, TNF-α increases the production of soluble FcγRIIa2 isoform which is produced by an alternative splicing of the transmembrane exon.[95] Alternatively, the increase of sFcγR levels could be related to an activation of proteases such as metalloproteases or serine proteases that have been shown to be involved in cleavage of FcγR.[96,97]

Mice bearing B cell tumors have 2-10 times more elevated sFcγR serum levels than their normal counterparts. Using an immunodot assay with the anti-mouse FcγR monoclonal antibody 2.4G2, the sFcγR levels have been measured in the sera of mice bearing syngeneic tumors of lymphoid and non-lymphoid origin.[98] These sera contain elevated amounts of sFcγR as compared to control mice. The enhancement of sFcγR levels was found to be independent on whether tumor cells express membrane-bound FcγR or not, suggesting that most of sFcγR are produced in response to tumors and originate from host rather than tumor cells. However, injection of mice with milligram amounts of purified mouse monoclonal IgG2a also provoked an increase of sFcγR serum levels, although to a lesser extent.[98] Thus, the increase of sFcγR in tumor-bearing mice has proven difficult to analyze: sFcγR can originate from both host cells and from FcγR$^+$ tumor cells and its release is triggered by the presence of a monoclonal paraprotein component. In mice bearing a variant hybridoma B cell tumor producing only the κ chain, we observed that sFcγR serum level exhibits an increase similar to that observed in mice bearing the parental IgG2a κ hybridoma B cell tumor (J.L. Teillaud and J. Moncuit, unpublished observation). Another difficulty lies in the fact that only one monoclonal antibody, 2.4G2, which does not discriminate between the different mouse FcγR isoforms, is available for performing such studies.

The role of membrane-bound and soluble FcγR in tumor progression has also been studied using an experimental model of tumors developing from BALB/c 3T3 cells transformed in vitro with Polyoma virus.[99-101] It has been shown that FcγRII is expressed by a subpopulation of Polyoma virus-transformed 3T3 cells after passaging in syngeneic animals as solid tumors. This expression decreases gradually when tumor-derived cells are explanted and grown in vitro. This phenomenon is observed again when explanted cells are inoculated into mice forming second-passage tumors.[99] Interestingly, these in vivo passaged FcγRII$^+$ transformed 3T3 cells exhibit a higher malignant phenotype than their FcγRII$^-$ clonal ancestors or their in vivo passaged FcγRII$^-$ counterparts.[100] Molecular analyses showed that it is the FcγRIIb1 isoform which is triggered upon passaging in vivo. In addition, experiments performed using Polyoma virus-transformed 3T3 cells transfected with

FcγRIIb1 cDNA showed that these cells exhibit a significantly higher tumorigenic phenotype than FcγR$^-$ neo^r transfected Polyoma virus-transformed 3T3 cells. Finally, inoculation of a mixture of FcγRIIb1$^+$ and FcγRIIb1$^-$ transformed 3T3 cells led to a dominance of FcγRIIb1$^+$ cells into the tumor-cell population over non-FcγRIIb1 expressor cells.[101] Thus, these data suggest that FcγR could play a major role in tumor progression in vivo. A possible mechanism could be due to the release of sFcγRII from these FcγRIIb1$^+$ polyoma virus-transformed 3T3 cells, creating an imbalance in the functions regulated by these molecules.[101]

Human soluble FcγR

FcR expression on peripheral blood cells and soluble FcγRIIa1/a2 (sCD32) and FcγRIII (sCD16) serum levels have also been studied in patients with multiple myeloma (MM).[11-13] It has been reported that FcR expression is increased on peripheral blood lymphocytes of these patients.[11] The type of FcR involved was related to the isotype of the secreted monoclonal paraprotein. For instance, only FcγR expression was increased in patients with MMγ, while patients with MMα exhibited higher levels of membrane-bound FcαR but not of FcγR as compared to healthy donors.[11] However, this study did not discriminate between FcγRII (CD32) and FcγRIII (CD16). A more recent study performed in our laboratory on seven MMγ patients using monoclonal antibodies that define cell subpopulations indicated the existence of a strong lymphopenia with a decrease in the absolute number of CD3$^+$ cells, associated with an inverted CD4/CD8 ratio in six of these patients.[12] Similarly, a marked decrease of the absolute number of CD16$^+$ and CD56$^+$ cells could be observed. However, both CD16$^+$ and CD56$^+$ cell percentages were not increased whatever the MMγ patient tested.[12] Thus, whether some MM patients show more elevated CD16 and/or CD32 expression on their lymphocytes remains to be clearly established.

It has been possible to analyze separately sCD32 and sCD16 serum levels in MM patients (Tables 7.3 and 7.4), as monoclonal antibodies which discriminate between these human receptors are available, making it possible to develop specific quantitative assays for FcγR evaluation.[12,70,71] sCD16 are produced both by neutrophils and NK cells, although the amount of sCD16 derived from these latter cells is negligible compared with the amount derived from neutrophils.[102,103] sCD16 serum levels have been analyzed in 165 MM patients, 29 patients with monoclonal gammopathies of unknown significance (MGUS), and 20 normal disease-free donors.[13] The level of sCD16 was found to be significantly decreased in sera from MM patients compared to sera from healthy and MGUS donors ($P = 0.0001$). In addition, a stage-dependent decrease in sCD16 was observed, with a highly significant difference ($P = 0.004$) between stage I and stage II+III MM patients (Durie-Salmon staging).[13] The correlation between the myeloma stage and the serum level of sCD16, which is related to the host response

(as myeloma cells express only type II FcγR, CD32) was also found more sensitive than that of β2-microglobulin, which reflects the tumor burden.[13] Interestingly, the decrease of sCD16 serum level was observed both in MMγ patients, in MMα patients, and in patients with Bence-Jones disease. By contrast to sCD16, MM patients showed no decrease of their soluble FcγRIIa1/a2 (sCD32) serum levels whatever the stage of the disease (F. Vely, unpublished observations).

The opposite modulation of FcγR serum levels observed in MM patients as compared with myeloma-bearing mice (decrease vs increase, respectively) has to be interpreted cautiously. First, both soluble FcγRII and FcγRIII serum levels were evaluated in mice with no discrimination between the two types of receptors. This quantitation reflects therefore both the tumor burden and the host immune response. By contrast, solely sCD16 (FcγRIII), which reflects the host immune response, were quantitated in MM patients. Second, the kinetics of the two diseases are different. The disease may have been first accompanied by the induction of FcγR, both in myeloma-bearing mice and in MM patients, due to the interaction with the monocloanl paraprotein, followed by a marked decrease as the disease progresses. However, the second step of the disease could be observed only in MM patients due to the rapid death of the mice. This hypothesis is strengthened by the fact that the injection in mice of monoclonal antibodies reacting both with FcγRII and FcγRIII initially induces an increase in cell-bound FcγR, followed by a decrease several days later.[92]

A decrease of sCD16 serum levels has also been reported in AIDS patients.[104] An initial increase of sCD16 serum level in clinical stage II and III (Staging of the Centers for Disease Control, CDC, 1990) is followed by a dramatic drop in patients with AIDS (stage IV). These changes correlated with the number of $CD4^+$ cells, the amount of p24 antigen in serum, and the anti-p24 antibody titers. Thus, it has been proposed that the sCD16 serum level could be a serum marker of HIV-related disease progression.[104] No specific changes in the number of $CD16^+$ (FcγRIIIa) natural killer cells was found, although there was a statistical correlation between the absolute number of $CD3^+/CD16^+$ cells and sCD16 serum levels.[104] Another study showed that a substantial population (25%) of neutrophils in patients with AIDS, AIDS-related complex and in HIV-1^+ intravenous drug abusers does not express FcγRIIIb (CD16).[105] No changes in the expression of FcγRII (CD32), CD11b, or DAF (also a PIG-anchored molecule) were found. Thus, it was concluded that the presence of a FcγRIIIb negative neutrophil population may be related to altered functions leading to common bacterial infections in advanced AIDS.[105] However, as previously mentioned, the non-expression of FcγRIIIb due to gene deletion does not lead to recurrent bacterial infections. Thus, whether this partial deficiency of FcγRIIIb expression on neutrophils in HIV-1 patients can account for bacterial infections has still to be demonstrated.

sCD16 and sCD32 serum levels have also been investigated in a number of other diseases (Tables 7.3 and 7.4). No major changes have been observed in 40 patients with B chronic lymphocytic leukemia (B-CLL).[71] When compared to sera from healthy donors, which appear to be extremely heterogeneous in their amount of sCD16, no significant modification was noted. A number of patients had very low levels, most of this group of patients having normal lymphocyte counts. The quantification of FcγRIIa2 (the soluble form related to membrane-bound FcγRIIa1/sCD32) in a group of 57 patients with B-CLL indicated that stage C (Binet's staging) patients exhibit a significant increase of FcγRIIa2 serum levels, compared to healthy donors, stage A and B patients, or patients with complete remission (A. Astier et al, submitted). This increase may be related to the tumoral mass or to a rapid lymphocyte count doubling time, although no significant difference has been demonstrated for these parameters between C versus A and B stages. FcγRIIa2 could originate from tumor B cells (as it has been reported that FcγRIIa transcripts are present in B cell lines representing different developmental stages)[106] or from cells belonging to the host immune system. The increase of FcγRIIa2 serum level in stage C patients could reflect a worsening of their clinical conditions triggering the release of cytokines such as TNF-α which has been involved in an increased production of FcγRIIa2.[95]

A retrospective study has been performed to evaluate sCD16 serum levels in 46 patients with acute myelogenous leukemia (AML), which is characterized by granulopenia and an increase in circulating myeloblasts and occasionally promyelocytes.[107] A significantly lower concentration of sCD16 was observed in the serum of AML patients by comparison with 48 age-matched normal donors (5.4 nM vs 9.5 nM, $p < 0.0005$). However, it is not known whether this decrease is due to granulocytopenia. In addition, the correlation of sCD16 serum levels with the clinical status of AML patients remains to be established.[107]

Analyses of sCD16 and sFcγRIIa (sCD32) serum levels in patients with an autoimmune-related pathogeny (such as systemic lupus erythematosus, SLE, Sjögren's syndrome, rheumatoid arthritis, Behçet's disease) or with inflammatory syndromes have been also conducted (Tables 7.3 and 7.4). Heterogeneous results have been obtained, which do not define the diagnostic and/or pronostic values of these two markers. Study of a group of 50 SLE patients indicated a slight increase of sCD16 in comparison with a group of 20 normal donors.[108] However, a detailed analysis showed that the group of SLE patients is very heterogeneous, with 11 patients only having a sCD16 serum level above the chosen cutoff (0.25 μg/mL). Furthermore, four SLE patients with sicca syndrome exhibited significantly lower sCD16 serum levels than the 46 remaining SLE patients.[108] It has also been suggested that sCD16 serum levels are higher in patients with primary Sjögren's syndrome, with a concomitant decrease in the percentage of FcγRIIIb$^+$ (CD16)

polymorphonuclear (PMN) cells. Diminished adherence and chemotaxis of PMN cells paralleled the decrease of FcγRIIIb expression.[109] The study of 33 patients with rheumatoid arthritis (RA) indicated an increase of sCD16 serum levels, which were inversely correlated with the mean fluorescence intensity (MFI) of FcγRIIIb$^+$ PMN cells labelled with anti-CD16 monoclonal antibodies.[110] By contrast, the density of CD11b (CR3) and CD35 (CR1) on these PMN cells was markedly increased. Synovial fluids from RA patients also contained higher levels of sCD16 compared to those observed in synovial fluids from non-RA patients.[110,111] sCD32 serum levels have been examined in our laboratory in a group of 108 SLE patients and of 32 patients with Behçet's disease (P. Ghillani and L. Musset, unpublished data). No significant difference was observed with a group of 103 healthy donors. However, 24 of the 108 SLE patients (22.2%) exhibited sCD32 serum levels higher than 90 ng/mL while only 7 of the 103 normal donors (6.8%) had sCD32 values above this level. Conversely, seven patients with Behçet's disease (21.8%) were found with sCD32 serum levels lower than 20 ng/mL by comparison with only 14.5% of the healthy donors. No clinical or biological correlations with known parameters could be defined in this study.

The role of sCD32 has been also examined using a recombinant purified molecule obtained by insertion of a termination codon 5' of sequences encoding the transmembrane domain of a human FcγRII cDNA.[112] The administration of recombinant sCD32 significantly inhibited the immune complex-mediated inflammatory response induced by the reversed passive Arthus reaction model in rats. The perivascular infiltrate and the red cell extravasation was less pronounced in the sCD32-treated group of animals. It has been also shown that recombinant sCD32 is a potent regulator of immune complex formation, delaying immune precipitation. However, it does not inhibit the complement-mediated prevention of immune precipitation, indicating that it does not block C1 binding to IgG containing immune complexes.[113] Thus, sCD32 could be a valuable therapeutic agent for the treatment of antibody or immune complex-mediated tissue damage.

sCD16 and sCD32 have also been found in other biological fluids such as saliva, urine and seminal fluid but at much lower concentrations than that found in serum.[67,111,114,115] In patients with adult respiratory distress syndrome (ARDS), levels of sCD16 in the bronchoalveolar lavage fluid were found five to seven times higher than that in healthy adults.[111] A significant decrease of sCD32 levels has been reported in saliva from patients with acute periodontitis when compared with periodontitis-free patients, while no change in sCD16 levels was observed.[114] It has been shown that sCD16 levels are lower in seminal plasma from patients with antisperm antibodies (ASA) than in ASA negative patients. This may be due to a steric interference from IgG, that could prevent sCD16 from modulating immunosuppression of antisperm immune responses observed in these patients.[115]

SOLUBLE FCεR AND PATHOLOGY

Soluble FcεRII (sCD23)

During the last decade, several studies have indicated that soluble FcεRII (also termed IgE-Binding Factors or sCD23) may regulate IgE production by interacting primarily with IgE-bearing B cells but also with other ligands (such as CD21) expressed on a variety of cell types.[86,87,116] sCD23 can also promote the proliferation of both anti-IgM activated normal B cells and Epstein-Barr virus (EBV)-transformed B cells, acting therefore as a B cell growth factor (BCGF).[116] Thus, sCD23 serum levels have been carefully evaluated in patients with B cell-related diseases (Table 7.4).

When 40 sera from patients with B-CLL were studied, all of them showed sCD23 serum levels three to 500-fold higher than in 24 controls.[117] With a few exception, sCD23 serum levels made it possible to differentiate B-CLL from other leukemia or lymphoma patients (acute lymphocytic leukemia, multiple myeloma, non-Hodgkin's lymphoma, hairy cell leukemia). In vitro studies indicated that B lymphocytes from B-CLL patients produced eight to 50 times more sCD23 than normal B cells. sCD23 levels correlated with the Rai staging of the disease and also, although weakly, with the lymphocyte count.[117] It has been proposed that sCD23 could be not simply a B-CLL marker but may also be involved in the proliferation of the leukemic B cells through an abnormal regulation of the expression of its two membrane-bound isoforms, FcεRIIA and FcεRIIB.[118-120] Elevated sCD23 serum levels has also been described in a retrospective study of AIDS patients before the appearance of acquired immunodeficiency syndrome (AIDS) associated-non-Hodgkin's lymphoma (NHL).[121] IgE serum levels have also been found significantly elevated in these patients. Thus, serum sCD23 may serve as a clinical tool for the early detection of AIDS-associated NHL and could represent a key-component in the lymphomagenesis seen in some AIDS patients.

Other studies have shown that elevated levels of sCD23 are also found in the serum of patients with several disease states associated with elevated IgE[122,123] or with enhanced B cell activation and humoral immunity such as Sjögren's syndrome, SLE, RA, autoimmune thyroiditis, myasthenia gravis, mixed connective tissue disease and allergies (Table 7.4).[122-127] By contrast, sCD23 serum levels were found to be significantly diminished in coeliac or Crohn's disease.[125] It has been shown that increased levels of sCD23 in RA patients are related to disease status by studying monozygotic twins discordant for RA.[128] Paired analysis showed significantly elevated sCD23 levels in affected twins when compared with their unaffected co-twins or normal controls. This increase was not related to disease duration.[128] Furthermore, in EBV-related disorders after liver transplantation with immunosuppression, plasma levels of sCD23 rapidly increased when clinical symptoms were evident (fever, lymphocyte infiltration in liver biopsy) and remained high, although the EBV hepatitis improved.[127]

Soluble FcεRI

The high affinity FcεRI has been produced in soluble form by transfecting cells with a cDNA encoding the extracellular domains of the α chain of human FcεRI.[129] The purified recombinant soluble FcεRI inhibits the binding of mouse IgE to FcεRI$^+$ cells, binds surface IgE expressed on B lymphoma cells in vitro and inhibits the passive cutaneous anaphylaxis model in vivo in Sprague-Dawley rats. Thus, these soluble molecules could represent potent regulators of type I hypersensitivity reactions. However, whether natural soluble α chain of FcεRI are present in sera of patients has not been yet documented.

MEMBRANE-BOUND AND SOLUBLE FcR: TOOLS FOR IMMUNOTHERAPY?

Targeting FcγR with Intravenous Immunoglobulin G (IVIG), Fcγ Fragments, or anti-FcγRIII Antibody

The targeting of FcγR in diseases where autoantibodies are suspected to play a key role has been developed in the early 1980s, when it became clear that intravenous infusion of polyclonal human IgG (IVIG) in children developing acute idiopathic thrombocytopenic purpura (ITP) refractory to coticosteroid treatments allowed to obtain transient or long-lasting responses.[130] It was postulated that the blockade of FcγR present on phagocytic cells could account for these beneficial therapeutic effects, at least in part. This possibility was reinforced by the finding that IVIG slowed the clearance of radiolabelled red cells coated with anti-D in four patients with ITP and supported by the success of anti-D in raising platelet counts in D$^+$ ITP patients.[131,132] The rationale of the treatment was that anti-D sensitized red cells would compete with IgG-coated platelets for binding to FcγR.[132] Further evidence of FcγR involvement was the efficacy of murine monoclonal antibody 3G8, directed against FcγRIII (CD16), in five of nine patients with refractory ITP and in one patient with ITP related to human immunodeficiency virus (HIV) infection.[133-135] However, the efficacy of IVIG treatment in ITP has also been related to idiotype/anti-idiotype interactions,[136] or to anti-infectious effect. These latter hypotheses have been seriously challenged, when it was shown that infusion of Fcγ fragments is also an efficient treatment of acute ITP in children.[137] Eleven of twelve children receiving Fcγ fragments (150 mg/kg daily on five consecutive days) showed a rapid increase in platelet counts to above the critical value of 50 x 10^9/L, thereby avoiding major hemorragic risk. Six children showed a stable response. While no sCD16 was detected in the Fcγ preparation used, sCD16 serum levels tested in five children showed transient or stable increases that correlated with the rise in platelet count.[137] Another recent assay confirmed the effectiveneess of Fcγ treatment in children with acute ITP, as the infusion of 300 mg/kg for only two consecutive days was equally efficient (Bonnet MC et al, submitted). In this trial, sCD16 serum levels

were found increased in 6 of the 13 children tested. By contrast, kinetics studies of sCD32 serum levels did not show any modification during the first month following treatment (M-C Bonnet, submitted).

The mechanisms by which Fcγ fragments or IVIG can trigger rapid and, at least in about half of ITP patients, stable responses remain unclear. The rapid increase of platelet counts following IVIG or Fcγ fragment infusions is likely due to the saturation of the different FcγR, thus preventing IgG-coated platelets from being captured and phagocytosized. However, one has still to understand how these IVIG or Fcγ fragments, infused under monomeric forms, can block FcγRI, already saturated by circulating endogeneous IgG, or bind to low-affinity FcγRII (CD32) or FcγRIII (CD16). Moreover, the stable responses observed are probably related to the pleiotropic effects triggered by FcγR/Fc interactions. For instance, it has been shown that the treatment of an ITP HIV^+ patient with the anti-CD16 monoclonal antibody 3G8 not only induces a long-term correction of thrombocytopenia but also, to a lesser extent, a stabilization of CD4 lymphocytes for 18 months, a stimulation of natural killer function and an elevation of tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ) and granulocyte-macrophage colony-stimulating factor (GM-CSF).[135] Similarly, the intravenous infusion of polyclonal human IgG in seven patients with stage III refractory MMγ has led to a disappearance of bone pains, an improvement of the performance status (Karnovsky), and an increase of the mean survival. sCD16 which were undetectable before therapy appeared in the sera from the seven patients and peaked after two to four weeks of treatment.[138] Thus, IVIG or Fcγ fragment infusion triggers a cascade of events, from changes in the network of membrane-bound FcγR and sFcγR to cytokine or inflammatory mediator release, with important immunoregulatory consequences.

All these data suggest that the use of molecules other than IVIG able not only to target FcγR but also to discriminate amongst the different FcγR types and isoforms is certainly a therapeutic approach to carefully consider both in autoimmune diseases and in other disorders as described below.

Interaction of anti-CD Antibodies with FcγR Via their Fc Region: Implications for Therapy

The use of monoclonal antibodies of IgG isotype should be carefully considered as it has been shown that the Fc region of these antibodies can trigger important secondary events through interaction with FcγR expressed by targeted or surrounding cells.

Anti-CD19 antibody and B cell malignancies

FcγRII (CD32) expressed on malignant B cells influences the modulation induced by anti-CD19 monoclonal antibodies.[139] An anti-CD19 IgG1 monoclonal antibody was found to induce modulation of CD19 antigens on Daudi cells more rapidly than did its IgG2a

switch variant. FcγRII did not co-modulate with CD19, but the anti-CD19 increased the capping and subsequent modulation of CD19 by increasing the calcium mobilization in these cells.[139] Thus, any therapy of patients with B cell malignancies based on the use of a monoclonal IgG antibody should carefully consider the IgG subclass to work with.

Anti-CD9 antibody and platelet aggregation

Activation of human platelets by IgG antibodies usually depends on their binding both to the target antigen and to FcγRII.[140] For instance, anti-CD9 monoclonal antibodies induce platelet aggregation only when their Fc region is bound to FcγRIIa1 (CD32), the only FcγR expressed on platelets.[141] Whether autoimmune disorders with anti-platelet IgG antibodies, leading to thrombocytopenia, involve such a mechanism remains to be elucidated. If so, specific targeting of FcγRIIa1 may be a way to prevent platelets from being activated by auto-antibodies. In addition, the activation of platelets by thrombin triggers the release of FcγRIIa2 (sCD32), containing the extracellular and intracellular regions of FcγRIIa1, but lacking the transmembrane domain, due to the alternative splicing of the transmembrane-coding exon.[142] FcγRIIa2 (sCD32) is likely to play an important role in the regulation of platelet activation by immune complexes or auto-antibodies, as its recombinant form competes efficiently with its membrane-bound counterpart, FcγRIIa1, for the Fc-dependent anti-CD9 antibody-induced platelet aggregation.[142]

Anti-CD3 antibody and immunosuppression in allograft transplantation

The use of the anti-CD3 antibody OKT3 as an immunosuppressive agent in clinical transplantation to prevent or to treat allograft rejection, has a major drawback, an associated cytokine release syndrome due to the binding of the Fc region of OKT3 to $Fc\gamma R^+$ surrounding cells. Recent studies showed that FcγR binding is not essential for attaining the immunosuppressive property of OKT3, but that the IgG subclass of the Fc region is essential to its acute toxicity and immunogenicity.[143,144] Chimeric antibody containing the mouse IgG3 region that does not bind mouse FcγR did not trigger cytokine production, TcR desensitization or humoral response, while still retaining potent suppressive properties in mice.[143] Thus, the generation of a chimeric anti-CD3 monoclonal antibody containing the Fc region of a human IgG subclass with poor affinity for human FcγR (i.e., IgG2 and/or IgG4) may be beneficial in clinical transplantation.

Targeting FcγR for Inducing Immune Suppression and/or Anergy

Targeting of FcγR by IgG-containing immune complexes can also induce suppression of immune responses. It has been shown that passively administered anti-Rhesus D (RhD) IgG antibodies to RhD^- women

immediately after delivery of an RhD$^+$ infant prevents Rh-immunization.[145] Furthermore, the sole prenatal treatment for severe Rhesus immunization is high-dose IVIG infusion.[146] The mechanisms by which the suppression of the anti-Rh immune response occurs are not elucidated and are a matter of debate. One could expect that the capture of anti-RhD sensitized red cells by phagocytic cells through FcγR would lead to increased presentation[44] and thus immunization. The opposite result is obtained in most treated women, with a major immunosuppression preventing any immunization. One possible basis for this immunosuppression is a cross-linking of various FcγR, or of FcγR with other FcR through IgG (and/or IgE or IgA present in trace amounts)-sensitized RhD$^+$ red cells. Recent data showed that cross-linking of FcεRI to low-affinity FcγRII inhibits IgE-induced release of mediator and cytokine.[8] Similarly, cross-linking of either BcR or TcR with FcγRII induces B cell[9,147,148] or T cell[9] inactivation, via the same tyrosine-based inhibition motif (ITIM) in the intra-cytoplasmic domain of FcγRIIb.[9] Another mechanism could be a release of sFcγR just after infusion, as reported in children with acute ITP treated with Fcγ fragments.[137] sFcγR (or IBF) have been shown, at least in vitro, to be involved in the immunosuppression of humoral responses[65] and thus could be to some extent be involved in this tolerization process.

Targeting FcγR with Bispecific Antibodies

A monoclonal antibody should bring together molecules or cells that mediate the desired biological effect to be effective as a therapeutic agent. However, many potentially useful antibodies are not of the appropriate isotype and are thus unable to activate human complement and/or to trigger FcγR on human cells, which are involved in effector functions such as antibody-dependent cell cytotoxicity (ADCC) and/or internalization of immune complexes followed by antigen presentation. One experimental approach developed over the last decade to overcome this problem has been the use of bispecific antibodies composed of one anti-FcγR antibody linked to an anti-target antibody.[149] This approach has been explored mostly with anti-FcγRI (CD64) and anti-FcγRIII (CD16) bispecific antibodies, with the development of phase I trials to determine the maximum tolerated dose (MTD) and to get information about the optimal biologic dose.

It has been shown that the targeting of HIV-1 to FcγR on human phagocytes via a bispecific antibody anti-FcγRI/anti-gp120 reduces in vitro the infectivity of HIV-1 to cells from a human T cell lymphoma line, H9.[150] The addition of IFN-γ-activated PMN cells or monocytes (thus expressing high levels of FcγRI/CD64) to cultures of HIV-1 plus H9 cells in presence of the bispecific antibody provoked a marked reduction of p24 levels, below those at culture initiation. This in vitro study indicates therefore that IFNγ-activated phagocytes can affect the natural course of HIV-1 infection of T cells, which could be of potential clinical importance. However, the possibility that FcγR could

also act as a enhancement factor of HIV infection in human cells has to be carefully examined. It has been reported that the addition of an anti-FcγRIII monoclonal antibody could inhibit HIV-1 and HIV-2 enhancement of infection of peripheral blood macrophages mediated by antibodies found in the blood of infected individuals and animals.[151]

Bispecific antibodies directed against FcγRI have also been developed for anti-tumor therapy. For instance, MDX-210, a bispecific antibody that binds simultaneously to FcγRI (outside the IgG binding site) and to HER-2/neu oncogene protein, was used in a phase Ia/Ib trial to determine the MTD.[152] Patients with advanced breast or ovarian cancer received a single intravenous infusion of MDX-210 at increasing dose levels from 0.35 to 10.0 mg/m^2. Treatment was well tolerated with malaise and low grade fevers. The maximum tolerated dose was 7.0 mg/m^2. The bispecific antibody could saturate FcγRI in a dose-dependent manner with up to 80% saturation at one hour. Plasma concentrations of TNF-α, IL-2 and G-CSF increased substantially after treatment. Monocytopenia occurred at 1-2 hours and resolved by 24 hours. One partial and one mixed tumor response were observed among 10 patients. However, decreased responses to MDX-210 were observed when some patients were challenged on days 8 and 15. Only weak monocytopenia and increased plasma cytokine levels were induced in that case. Human anti-mouse antibodies (HAMA) developed within 15 days in two patients, which could account, at least partly, for this desensitization.[152]

Similarly, a bispecific antibody against FcγRIII (CD16) and to HER-2/neu, 2B1, has been developed and tested in a phase-I trial.[153] Fifteen patients with c-erbB-2 overexpressing tumors were treated with 1 hour intravenous infusions of 2B1 on days 1, 4, 5, 6, 7, and 8 of a single course treatment. Doses were 1.0, 2.5 and 5.0 mg/m^2. Toxicities included fevers, rigors, nausea, vomiting, and leukopenia. The only dose-limiting effect was thrombocytopenia in two patients at the 5.0 mg/m^2 dose level. These patients had received extensive prior myelosuppressive chemotherapy. The initial treatment induced a strong increase of TNF-α, IL-6, IL-8, and, to a lesser extent, of GM-CSF and IFN-γ serum levels. HAMA were induced in 14 of 15 patients. The binding property of 2B1 appeared to be retained in vivo, as it bound to all neutrophils and to a proportion of monocytes and lymphocytes. Several minor clinical responses were observed (resolution of pleural effusions and ascites, of one liver metastasis; reduction of the thickness of chest wall disease).[153] Thus, this trial indicates that treatment with anti-FcγRIII/anti-target antigen bispecific antibodies has potent immunological consequences that could help to trigger anti-tumor functions via FcγRIII$^+$/CD16 cells in cancer patients.

The effectiveness of therapeutic approaches involving bispecific antibodies is likely to be improved in the near future. On the one hand, efforts to prevent the patients from developing HAMA have been

made either by the construction of humanized anti-FcγRI[154] or FcγRIII (Dr Ring DB, Antibody Engineering meeting, La Jolla, Calif. USA, December 7-9, 1994) antibodies or by the screening of combinatorial human phage-displayed libraries to isolate recombinant antibody fragments (Fab or single chain Fv, scFv) (R. Kontermann et al, unpublished data). On the other hand, approaches to increase expression and/or functional activities of FcγR expressed by effector cells before or during bispecific antibody infusions have been explored. For instance, an elevated FcγRI expression is observed on neutrophils isolated from G-CSF-treated cancer patients.[155,156] ADCC assays demonstrated that these FcγRI$^+$ neutrophils are potent anti-tumor effector cells, at least in vitro, and can be recruited by bispecific antibodies directed to FcγRI and to tumor antigens (HER-2/neu, disialoganglioside, G_{D2}).[155,156] Based on these observations, a phase I study has been developed to explore the toxicity of a G-CSF and anti-FcγRI/anti-HER-2/neu (MDX-210) bispecific antibody combination.[155] In this study, patients receiving G-CSF were treated with escalating doses of MDX-210. Some patients had transient fever and short periods of chills related to elevated plasma levels of IL-6 and TNF-α. A transient decrease of the absolute neutrophil count was also observed, as well as that of total white cell blood count. During G-CSF application, isolated neutrophils were highly cytotoxic in the presence of MDX-210 in vitro.[155] The concomitant infusion/injection of anti-FcγRIII/anti-target antigen bispecific antibody and IL-2 (that provokes an increase of FcγRIII/CD16 expression) should also be evaluated in the near future. Thus, the combined administration of a bispecific antibody against a particular FcγR and a given cytokine that upregulates this receptor may be an efficient therapeutic approach to trigger strong tumor lysis by effector cells in vivo. FcγRI targeting could be also invaluable for triggering humoral response against tumor cells. It has been recently reported that antigen targeting to this receptor, which is specifically expressed on myeloid cells, triggers enhanced antibody responses in transgenic human FcγRI/CD64 mice immunized with an anti-human FcγRI antibody containing antigenic determinants.[157]

Targeting FcεR

Targeting FcεRI

Blockade of human FcεRI expressed on mast cells, basophils and epidermal cells could be an efficient approach for the therapy of immediate hypersensitivity reactions. One possible approach that has been evaluated is the use of peptides derived from the human ε chain of IgE and competing with IgE for FcεRI binding.[158] A recombinant peptide corresponding to residues 301-376 at the junction of constant regions 2 and 3 of the human IgE ε chain was found to block the in vivo passive sensitization of human skin mast cells and in vitro sensitization of

human basophils with human IgE antibodies. However, approximately 11- to 13-fold higher concentration of the recombinant peptide than IgE myeloma protein was required for 50% inhibition of antigen-induced histamine release.[158] The design of other peptides competing also with IgE for binding to FcεRI has not been successful enough to develop therapeutic trials using this strategy so far.

Targeting FcεRII/CD23 in collagen-induced arthritis

As described above, increased serum levels of sFcεRII/sCD23 have been observed in patients with rheumatoid arthritis (RA). Thus, the effects of neutralizing anti-CD23 antibodies have been evaluated in a mouse model of type II collagen-induced arthritis.[159] Successful disease modulation was achieved by treatment of arthritic mice with either polyclonal or monoclonal antibodies. A dose-related amelioration of arthritis was achieved, with significantly reduced clinical scores and number of affected paws. A marked decrease in cellular infiltration as well as limited destruction of cartilage and bone were also evident in anti-CD23-treated animals.[159] Thus, CD23 appears to be involved in a mouse model of human RA and may be a good target for therapeutic human trials.

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