A full complement of receptors in immune complex diseases

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In this issue of the *JCI*, Shushakova et al. (1) describe experimental results that provide a direct link between the C5a anaphylotoxin and the IgG Fc receptors (FcγRs) in mediating immune complex–triggered (IC-triggered) inflammatory disease. Using a murine lung model of IC-induced inflammation, the authors demonstrate that C5a, acting through the C5a receptor (C5aR), exacerbates inflammation in part by altering the ratio of activation to inhibitory FcγR expression on alveolar macrophages, enhancing the former and suppressing the latter, thereby optimizing the ability of alveolar macrophages to respond to ICs and trigger cytokine release and neutrophil chemotaxis. C5a now joins the ranks of other regulators of FcγR expression, such as IFN-γ and intravenous immunoglobulin (IVIG), in modulating IC-induced inflammation by acting on the primary IC targets, the FcγRs. What makes this observation worthy of comment is the historical context of IC-mediated inflammation and the confusion that has surrounded the basic mechanism of this fundamental immunological reaction, the Arthus reaction.

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Nonstandard abbreviations used: IgG Fc receptor (FcγR); immune complex (IC); C5a receptor (C5aR); intravenous immunoglobulin (IVIG); immunoreceptor tyrosine-based activation motif (ITAM); tyrosine-based inhibitory motif (ITIM); glucose-6-phosphate-isomerase (GPI).

The Arthus reaction, 1903–1996

In 1903 Maurice Arthus published his observations on the induction of a localized inflammatory reaction at the site of repeated immunizations with a foreign antigen (2). The Arthus reaction, as it has become known, results from the deposition of ICs in specific anatomic sites and the subsequent activation of inflammatory responses to these complexes. This reaction has served as the basis for dissecting the cellular and molecular events that are triggered by IC deposition and serves as the basis for our understanding of the pathophysiology of IC-mediated diseases, such as lupus and rheumatoid arthritis. While the role of antibody-antigen complexes is clearly established in the inflammatory response, the triggers and mediators downstream of ICs have been more difficult to identify with certainty. The detailed in vitro observations that ICs bind early components of complement and result in their activation, leading to the generation of C3 as a central protein in complement activation, led to the widely held model of the mechanism of IC-mediated inflammation. In this model, ICs directly lead to C3 activation followed by the formation of the late components of complement activation, notably C5a and the membrane attack complex. ICs, acting through complement pathways, were thus deemed to be responsible for all the downstream effector responses, including edema, hemorrhage, neutrophil infiltration, and the release of pro-inflammatory mediators such as
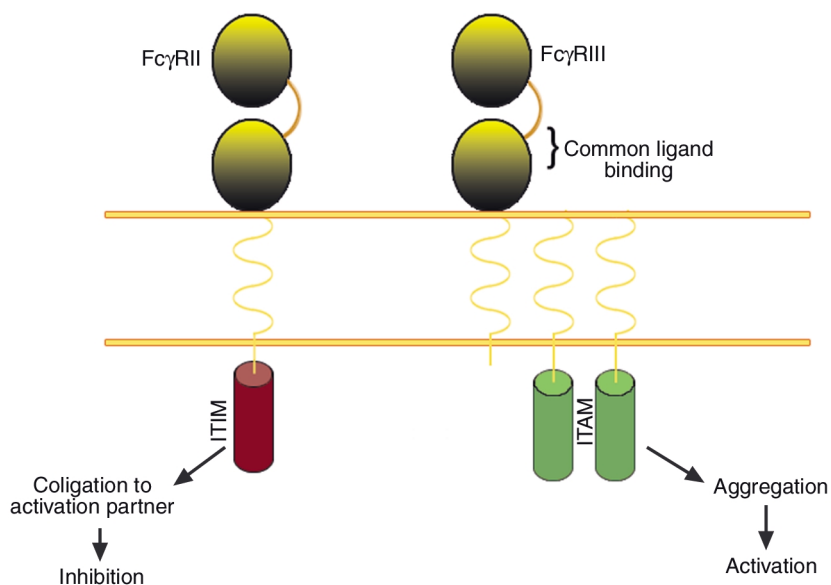
Figure 1

IgG Fc receptors are expressed as activation/inhibitory pairs. A schematic representation of an activation receptor, FcγRIII, and its inhibitory receptor pair, FcγRII, highlight the basic principle that the interaction of two receptors with common ligand binding domains that mediate opposing signals will set a threshold for stimulation.

TNF and IL-1 (3). The authors of these models did not contemplate the possibility of direct activation of effector cells by ICs, mediated by the cellular receptors for these complexes, the FcγRs (4). These cell surface receptors had been shown to be potent activators of effector cells upon their crosslinking by ICs. A unique aspect of the FcγRs was their heterogeneity, in which a conserved ligand-binding domain was associated with alternative intracytoplasmic signaling motifs (5) (Figure 1). These motifs could either activate cellular responses through the immunoreceptor tyrosine-based activation motif (ITAM) sequences or inhibit the activation response through immune receptor tyrosine-based inhibitory motif (ITIM) sequences. Activation and inhibitory FcγRs were found to be coexpressed on effector cells such as mast cells, macrophages, and neutrophils (6), thus leading to the hypothesis that the ratio of these opposing signaling receptors was critical in setting thresholds for the inflammatory activity of ICs (7).

## Mouse knockouts redefine the Arthus reaction

Critical evaluation of the roles of these alternative pathways became possible with the advent of targeted gene disruption in the mouse in the 1990s. Disruption of activation FcγRs, notably FcγRIII, resulted in ablation of IC-mediated inflammatory responses, as well as the ability of cytotoxic antibodies to trigger in vivo clearance of opsonized cells or mediate antibody-dependent cellular cytotoxicity in vivo (8). Conversely, disruption of the inhibitory FcγRIIB receptor led to enhancement of IC-mediated inflammation and antibody-triggered cytotoxicity and clearance of opsonized cells in vivo (9, 10). What of the role of complement then? Using the reverse passive Arthus reaction as a model for IC-triggered inflammation, researchers found that mice deficient in the classical, early components of complement, including the central activator C3, were able to mount a normal inflammatory response to ICs (11). C3-deficient mice, in general, where found to have a wild-type response to cytotoxic antibody–triggered inflammation and opsonized cell clearance (8, 12). In active models of IC deposition, such as anti-glucose-

6-phosphate-isomerase (GPI) arthritis, a partial dependence on C3 was observed, consistent with its role in stabilizing the IC (13). In contrast, loss of activation FcγRs ablated the inflammatory response in all models of IgG-induced inflammation, while loss of the inhibitory FcγR enhanced the response. Further studies went on to demonstrate that it was the activation FcγRIII receptor on mast cells that initiated the Arthus reaction, thus putting FcγRIII upstream of cytokine release and neutrophil influx (14, 15). In contrast, the late component C5a was found to have a variable effect on IC-mediated inflammation in different experimental models, ranging from an essential role in the anti-GPI arthritis model to a modest one in IC alveolitis, suggesting that the specific details of each model, such as the strain background, could affect the outcome (15).

Convergence of pathways

How are we to understand the roles of FcγRs and complement in the IC-induced inflammatory response? The most consistent model that emerges from the genetic data of the last six years is the absolute requirement for

engagement of activation FcγRs by ICs. This activation is modulated by the expression ratio of activation and inhibitory FcγRs. Early complement components, including C3, are likely to be involved in IC clearance and stability and will thus play variable roles in induced models of IC-mediated inflammation. The late components, such as C5a, behave as downstream cytokines, activated by the triggering of FcγRs on effector cells such as mast cells. In this model, the essential step is the regulation of FcγR activation, and not complement. FcγR regulation, in turn, can be thought of in terms of the independent modulation of the activation and inhibitory receptors. This level of regulation has been observed in the developmental expression of these receptors on myeloid cells. Mast cells, macrophages, and dendritic cells, when they emerge from the bone marrow, primarily express the inhibitory FcγRIIB, with little expression of the activation FcγRIII, thus providing a simple mechanism to prevent inappropriate activation of these circulating cells by ICs (16). However, this ratio inverts in an inflammatory environment, where

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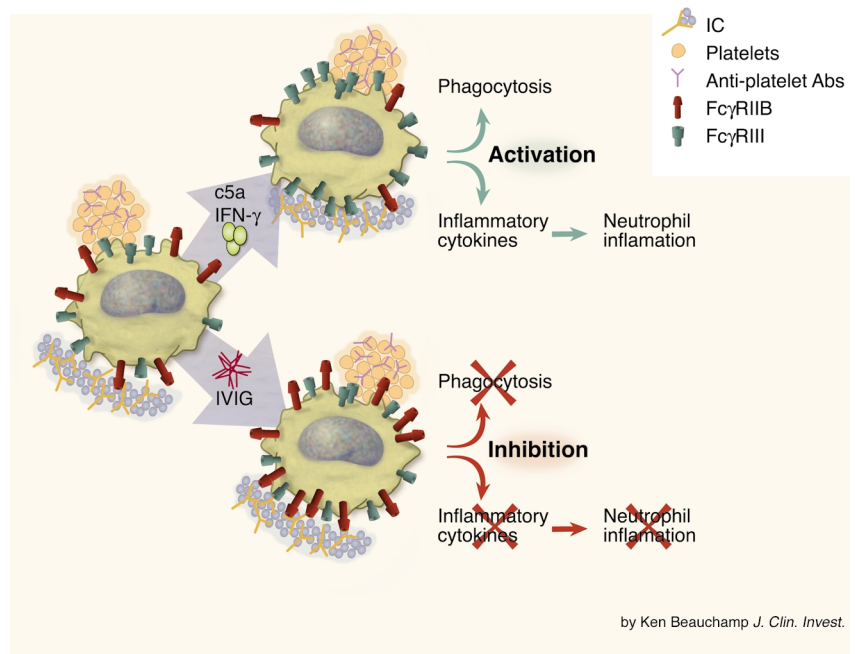
Figure 2
Regulating expression of the IgG Fc RII/RIII ratio modulates inflammatory responses. An effector cell, shown here as a macrophage, expresses both activation and inhibitory FcγRs and is capable of responding to IC or cytotoxic antibody stimulation at a defined threshold. Upregulation of the activation FcγRIII, induced by IFN-γ or C5a, results in a lowered threshold for IC stimulation and consequently an enhanced inflammatory response. Conversely, upregulation of the inhibitory FcγRII molecule by IVIG raises the threshold for IC stimulation and suppresses inflammatory responses to IgG antibodies.

local production of cytokines such as IFN-γ and TNF result in the upregulation of FcγRIII and downregulation of FcγRIIB (17). To this list of cytokines that regulate FcγR expression we can now add C5a through C5aR, based on the results reported in this issue of the *JCI* by Shushakova and colleagues (1). The authors demonstrate that the ability of C5a to upregulate FcγRIII and downregulate FcγRIIB accounts for its ability to augment IC-mediated inflammation and may also explain the variability in the data on the role of C5a in various IC-induced models of inflammation. FcγR expression is under the redundant control of numerous other cytokines as well as genetic background effects; thus specific strains and models will likely have variable dependence on a specific cytokine pathway. Once maximal levels of the activation/inhibitory ratio are achieved, further augmentation is not likely to modify the inflammatory response.

From the preceding it is apparent that enhancing inhibitory receptor expression should protect the organism from IC-mediated inflammation. This prediction has been realized in the observation that the anti-inflammatory activity of IVIG results from its ability to upregulate expression of FcγRIIB, thereby raising the threshold required for ICs to trigger FcγRIII activation (18). The cytokine responsible for this upregulation has not been identified, but it is likely to fall within the class of anti-inflam-

matory cytokines. These reciprocal effects on FcγR activation and antibody-triggered inflammation are summarized in Figure 2.

Our understanding of the molecular mechanisms that underlie the pathophysiology of IC-triggered diseases has focused attention on the central role of the IgG Fc receptors and the secondary role of complement in these in vivo reactions. FcγRs exert their function through the paired expression of activation and inhibition receptors. The ratio of these opposing receptors is therefore the critical factor in determining whether an antibody response will result in inflammatory disease or quiescence. The identification of this critical target for antibody-mediated effects in vivo permits the identification of pathways that modulate these cellular targets. Thus, the factors that regulate this ratio will be central to defining the homeostatic mechanisms that function to direct the inflammatory response to protect the host and prevent inappropriate activation and unbridled inflammatory states. This will be a fertile area for future investigations.

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