

# A Dominant Role for Mast Cell Fc Receptors in the Arthus Reaction

Diana L. Sylvestre and Jeffrey V. Ravetch\*

Division of Molecular Biology  
Memorial Sloan-Kettering Cancer Center  
1275 York Avenue  
New York, New York 10021

## Summary

Antibody-antigen complexes are central to the inflammatory response and are implicated in the development of such diverse diseases as systemic lupus erythematosus, rheumatoid arthritis, immune glomerulonephritis, and vasculitis. We recently demonstrated that experimental immune complex-mediated injury in mice, as modeled by the cutaneous Arthus reaction, requires receptors for the Fc portion of the antibody and is unaffected by deficiencies in complement components. However, the responsible cell type(s) and Fc receptor(s) were not known. We now demonstrate by differential reconstitution *in vivo* that Fc $\gamma$ RIII on mast cells is necessary for this inflammatory response. We propose a general model of antibody-mediated diseases as an immunopathologic spectrum whose specific manifestations are determined by the Fc receptor and cell type engaged.

## Introduction

The molecular mechanisms by which immune complexes trigger such diverse diseases as systemic lupus erythematosus, rheumatoid arthritis, immune vasculitis, and glomerulonephritis remain poorly understood. These macromolecular complexes are able to interact directly with effector cells through Fc receptors (Fc $\gamma$ Rs) or indirectly through the binding and activation of complement components. This promiscuity of immune complex binding and the heterogeneity of immune complex receptors have confounded attempts at defining the sequence of events that culminates in immune complex-triggered injury.

Fc $\gamma$ Rs form a key link between the humoral and cellular immune systems. They are expressed by a wide variety of hematopoietic cells and can bind either monomeric immunoglobulin G (IgG) (the high affinity receptor Fc $\gamma$ RI) or immune complexes (the low affinity receptors Fc $\gamma$ RII and Fc $\gamma$ RIII). These receptors share structurally related ligand-binding domains, but differ in their transmembrane and intracellular domains. In addition, both Fc $\gamma$ RI and Fc $\gamma$ RIII share with the IgE-binding receptor Fc $\epsilon$ RI their association with the  $\gamma$  subunit of the Fc $\gamma$ R complex, which is necessary for assembly and signaling.

The availability of defined mutations in mice that perturb immune complex interactions with effector cells has facilitated a dissection of the pathways involved in immune complex injury. Mice genetically deficient in the

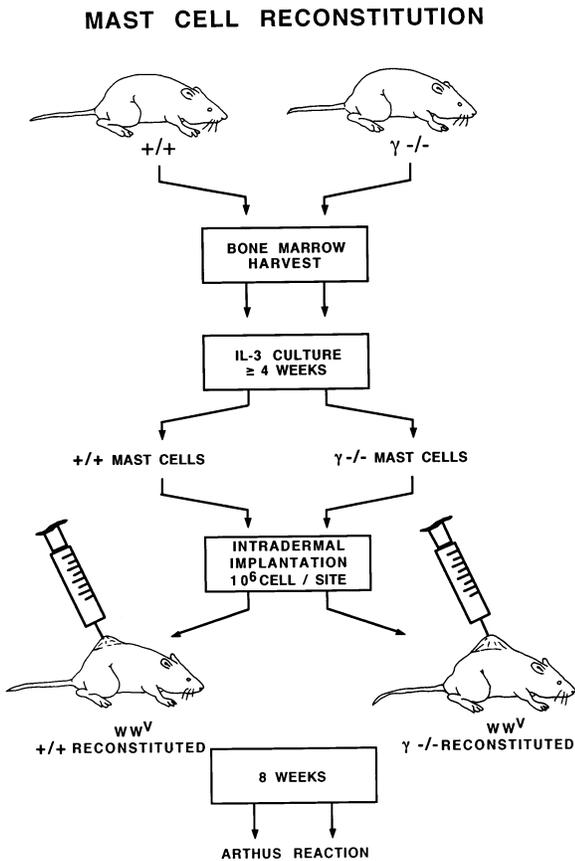
$\gamma$  subunit of the Fc $\gamma$ R complex, and lacking functional Fc $\gamma$ RI and Fc $\gamma$ RIII as well as the high affinity receptor for IgE, Fc $\epsilon$ RI (Takai et al., 1994), exhibit a grossly diminished inflammatory reaction in the reverse passive Arthus reaction (Sylvestre and Ravetch, 1994). In contrast, mice genetically deficient in complement component C3, as well as those deficient in C4 or C5, exhibit Arthus reactions indistinguishable from wild-type animals (Sylvestre et al., 1996). Thus, the presence of an intact complement cascade is neither necessary nor sufficient to trigger or propagate the Arthus reaction, indicating instead an essential requirement for Fc $\gamma$ R engagement, resulting in cellular activation, chemotaxis, and infiltration of neutrophils. The Fc $\gamma$ R-bearing cell types residing in the skin that most likely mediate this response are the mast cell or the epidermal Langerhans cell, both of which express Fc $\gamma$ RII and Fc $\gamma$ RIII, or tissue macrophages, which express Fc $\gamma$ RI, Fc $\gamma$ RII, and Fc $\gamma$ RIII (Ravetch and Kinet, 1991; Esposito-Farese et al., 1995). Previous studies have shown that the mast cell-deficient mouse line, *W/W<sup>v</sup>*, exhibits an attenuated Arthus reaction, which is reconstituted by mast cell replacement. We therefore proposed that the mast cell Fc $\gamma$ RIII was the molecule responsible for triggering the inflammatory response to IgG immune complexes (Sylvestre and Ravetch, 1994).

## Results and Discussion

To test this hypothesis, we developed a protocol based on differentially reconstituting the mast cell-deficient mouse strain *W/W<sup>v</sup>* with mast cells derived from wild-type or Fc $\gamma$ R-deficient mice. As shown in Figure 1, bone marrow from  $\gamma$   $-/-$  mice and littermate  $+/+$  controls was harvested, cultured under conditions that result in homogeneous population of mast cells, and implanted intradermally into *W/W<sup>v</sup>* recipient animals. Previous studies have shown that the migration of mast cells to their normal locations in the postcapillary venules, as well as the alteration to a characteristic skin cell phenotype, occurs slowly over a period of approximately 8 weeks (Nakano et al., 1985; Kitamura, 1989; Galli, 1990). Therefore, after waiting 8 weeks for full reconstitution, we performed the reverse passive Arthus reaction on reconstituted mice, using  $\gamma$   $-/-$ , wild-type C57BL/6, and *W/W<sup>v</sup>* C57BL/6 mice as controls, as described previously (Sylvestre and Ravetch, 1994).

Figure 2 shows representative skin sections harvested from an 8 hr Arthus reaction; the purpuric spots of the hemorrhagic reaction are characteristic of this inflammatory response.  $\gamma$   $-/-$  mice (Figure 2A) show little detectable response, whereas  $+/+$  control mice (Figure 2B) exhibit vigorous hemorrhage. The baseline *W/W<sup>v</sup>* reaction (Figure 2C) is reduced by approximately 50%, as has been previously reported (Zhang et al., 1991). Reconstituting the *W/W<sup>v</sup>* mice with mast cells derived from  $\gamma$   $-/-$  animals (Figure 2D) does not result in any further augmentation of the Arthus response of the recipient *W/W<sup>v</sup>* mice, while reconstitution with  $+/+$  mast

\*Present address: The Rockefeller University, 1230 York Avenue, New York, New York 10021.



**Figure 1. Protocol for Differential Reconstitution of Mast Cells**  
Bone marrow was harvested from  $\gamma -/-$  or  $+/+$  littermate controls and cultured separately in WEHI-conditioned medium as a source of IL-3 for 4–6 weeks, resulting in a homogeneous population of immature mast cells.  $W/W^v$  mast cell-deficient mice were then injected intradermally in two separate sites with  $1 \times 10^6$  mast cells per site, and the Arthus reaction was performed after an incubation period of 8 weeks to allow for mast cell migration and phenotypic differentiation.

cells results in substantial enhancement of the Arthus reaction (Figure 2E), approximately to the levels of  $+/+$  controls in the reconstituted areas.

The Arthus reaction can be dissected into three distinct components: a rapidly evolving edema reaction, followed by the more slowly emerging hemorrhagic and infiltrative phases of the reaction. Microscopic quantitation of the three parameters of edema, hemorrhage, and neutrophil infiltration in an 8 hr Arthus reaction, which we have previously shown to correlate well with other means of quantitation (including  $^{125}\text{I}$ -labeled human serum albumin for edema and the colorimetric measurement of myeloperoxidase), is shown in Figure 3 (Sylvestre and Ravetch, 1994). The left group of bars represents the edema seen at this timepoint, the central group represents hemorrhage, and the right group represents neutrophil infiltration. As reported previously,  $+/+$  inflammation is significantly greater than that of  $-/-$  for the three parameters (Sylvestre and Ravetch, 1994). In addition, the edema, hemorrhage, and infiltration exhibited by the  $W/W^v$  mice is intermediate between these

two genotypes, although some statistical overlap occurs for each of the criteria. Reconstituting  $W/W^v$  mice with  $+/+$  mast cells restores the edema, hemorrhage, and neutrophil infiltration to levels similar to that of wild-type control animals, as has been previously reported (Zhang et al., 1991). However, the inflammation exhibited by the  $-/-$  mast cell-reconstituted mice is significantly less in all three criteria, not differing significantly from that of the background  $W/W^v$  genotype. This was not due to a difference in density of reconstituted mast cells between the two genotypes: microscopic quantitation of the number of mast cells per  $\text{mm}^2$  showed a density of  $11 \pm 3$  for  $+/+$  and  $10 \pm 3$  for  $-/-$ .

The finding that mast cell  $\text{Fc}\gamma\text{RIII}$  is responsible, in part, for the triggering of the Arthus reaction underscores the similarity between the response to IgG immune complexes and the IgE-mediated anaphylactic reaction, in which  $\text{Fc}\epsilon\text{RI}$  cross-linking on mast cells results in an acute inflammatory response. Thus, despite the long-held dogma that these two reactions are fundamentally different, these data support the conclusion that they are mechanistically quite similar. It is worth noting that in the original description of the Arthus reaction, the response was called “an anaphylactic response of the rabbit to horse serum” (Arthus, 1903).

The Arthus reaction and the anaphylactic reaction differ, however, in their kinetics of response and histological appearance. The edema triggered by IgG immune complexes is more gradual in onset than that elicited by cross-linking IgE. In addition, the late phase Arthus reaction exhibits not only cellular infiltration, as seen with IgE triggering (Wershil et al., 1991), but also hemorrhage and, in severe cases, necrosis due to damage to the vascular wall. What then accounts for these physiological differences? The relevant  $\text{Fc}\gamma\text{Rs}$  differ only in their  $\alpha$  subunits, which may account for differential signaling from the same cell in response to ligand cross-linking. Alternatively, the differences between these two reactions may reflect the contribution of additional, uncharacterized components in one that are absent in the other. In addition, our data demonstrate that the mast cell-deficient mouse has an Arthus reaction intermediate in intensity between the wild-type and  $\gamma -/-$  mice. The partial response seen in the mast cell-deficient mouse, which is totally abolished in the  $\gamma -/-$  mouse, argues for the involvement of another  $\text{Fc}\gamma\text{R}$ -bearing cell type that is required for reconstitution of the complete reaction. Tissue macrophages or Langerhans cells are  $\text{Fc}\gamma\text{RIII}$  positive and could function to mediate the later phases of the Arthus reaction, perhaps thereby contributing to the diversity of syndromes seen in IgG-mediated inflammatory disease states.

The establishment of parallels between IgG- and IgE-mediated reactions suggests the interesting possibility that antibody-mediated diseases may represent an overlapping immunopathologic spectrum whose manifestations are dictated by the characteristics and distribution of the  $\text{Fc}\gamma\text{R}$  engaged. An understanding of the different pathways elicited by IgE and IgG when signaling through their respective mast cell  $\text{Fc}\gamma\text{Rs}$ , as well as the cell types that contribute to the different reactions, should provide valuable insight into the process of inflammation.

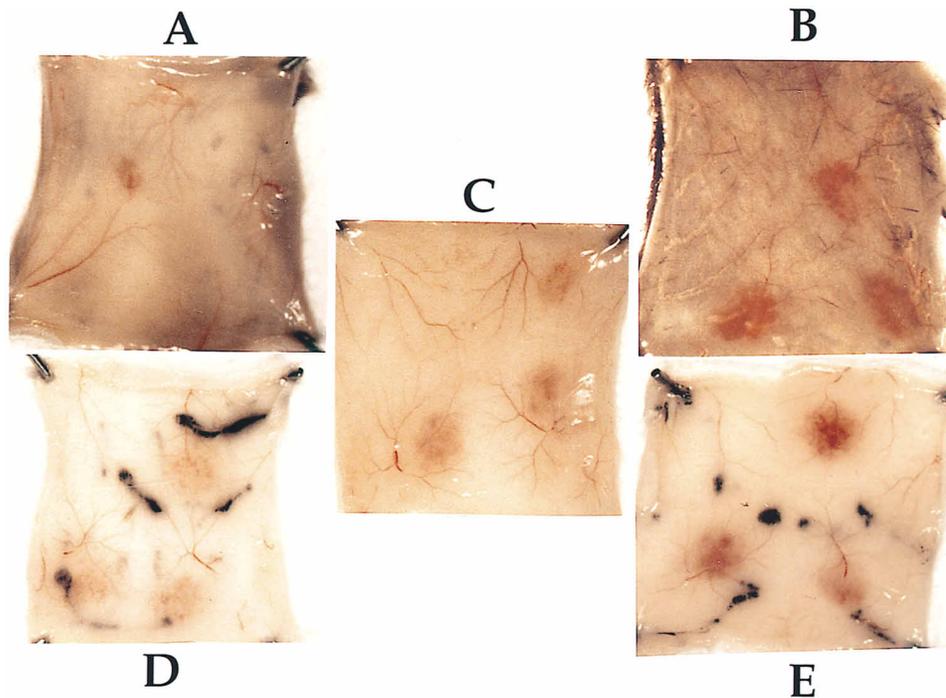


Figure 2. Hemorrhage from 8 hr Arthus Reaction

Inverted skin samples are shown from control  $\gamma^-/-$  (A),  $+/+$  (B), and unreconstituted  $W/W'$  mice (C).  $W/W'$  mice were reconstituted with either  $\gamma^-/-$  mast cells (D) or  $+/+$  mast cells (E) in the upper right and lower left positions, as marked by tattoos. Negative control injections were given in the upper left position, and 30  $\mu\text{g}$  of anti-ovalbumin was used in the other three sites; the dorsal skin was harvested 8 hr after intravenous administration of 20 mg/kg ovalbumin. Results are representative of at least six mice in each group.

#### Experimental Procedures

##### Mast Cell Reconstitution

Mice were sacrificed using CO<sub>2</sub> asphyxiation, and bone marrow was flushed from femurs using sterile phosphate-buffered saline. Cells were resuspended at a density of  $3 \times 10^5$  in RPMI, 10% FCS supplemented with 15% WEHI-conditioned medium as a source of interleukin-3 (IL-3). Nonadherent cells were harvested weekly and resuspended in the same medium at a density of  $1 \times 10^5$ . After 4–8 weeks of culture, the mast cells were washed and resuspended in phosphate-buffered saline at a density of  $1 \times 10^6$  per 100  $\mu\text{l}$  and immediately injected intracutaneously into the shaved dorsal skin of  $W/W'$  mice in a discrete bleb, with two separate sites per animal.

Sites of implantation were marked with india ink tattoos. Both reconstituted genotypes contained approximately equivalent numbers of mast cells as assessed histologically. Mast cell density was quantitated by counting mast cell numbers in serially cut Giemsa-stained skin sections, as previously described (Walker et al., 1985).

##### Arthus Reaction

Mice were anesthetized with avertin and their backs were shaved. We intradermally injected 30  $\mu\text{g}$  of either preimmune polyclonal rabbit IgG or polyclonal rabbit IgG against ovalbumin in a volume of 7.5  $\mu\text{l}$  in a discrete spot, followed immediately by 20 mg/kg ovalbumin. Mice were sacrificed using CO<sub>2</sub> asphyxiation at either 2 or

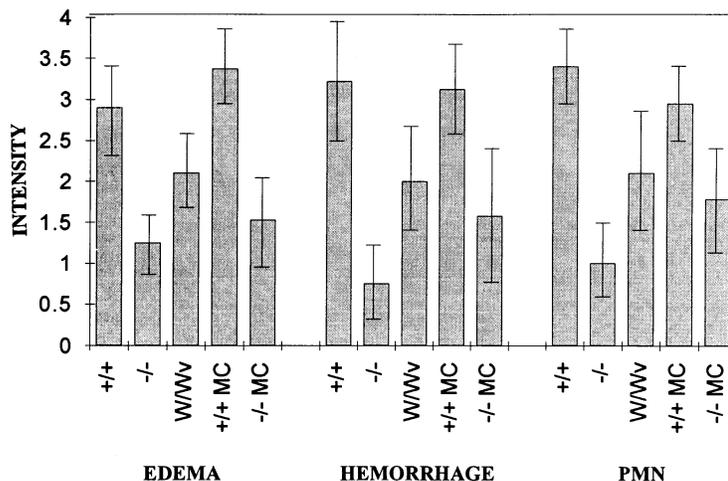


Figure 3. Microscopic Quantitation of the 8 hr Arthus Reaction

Histological scoring was based on a 1+ (minimal) to 4+ (vigorous) scale for the parameters of edema, hemorrhage, and neutrophil infiltration (PMN) 8 hr after a standard Arthus reaction using 30  $\mu\text{g}$  of antibody. Abbreviations are as follows:  $+/+$ , wild type;  $-/-$ ,  $\gamma$  knockout;  $W/W'$ , unreconstituted mast cell deficient;  $+/+$  MC,  $W/W'$  reconstituted with wild-type mast cells;  $-/-$  MC,  $W/W'$  reconstituted with  $-/-$  mast cells. Results are based on at least 16 sections in each group; error bars are  $\pm$  SD. The differences between the reconstituted mice were statistically significant for each of the parameters of edema ( $p = 0.008$ ), hemorrhage ( $p = 0.018$ ), and neutrophil infiltration ( $p = 0.048$ ), as determined by the paired Student's *t* test.

8 hr, and dorsal skins were harvested. Skin sections were fixed in 10% buffered formalin and stained with hematoxylin and eosin.

Hematoxylin–eosin-stained skin sections were graded in a blinded fashion for each of the parameters of edema, hemorrhage, and neutrophil infiltration on a scale of 1+ (minimal) to 4+ (vigorous) at both 2 hr and 8 hr.

#### Statistical Analysis

The differences in mast cell counts, as well as a comparison of the values for the different Arthus reaction parameters between genotypes, were analyzed for statistical significance ( $p < 0.05$ ) using the paired Student's *t* test.

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#### References

- Arthus, M. (1903). Injections répétées de sérum de cheval chez le lapin. *C.R. Soc. Biol.* 55, 817.
- Esposito-Farese, M.-E., Sautes, C., de la Salle, H., Latour, S., Bieber, T., de la Salle, C., Ohlmann, P., Fridman, W.H., Cazenave, J.-P., Teillaud, J.-L., Daeron, M., Bonnerot, C., and Hanau, D. (1995). Membrane and soluble Fc $\gamma$ RII/III modulate the antigen-presenting capacity of murine dendritic epidermal Langerhans cells for IgG complexed antigens. *J. Immunol.* 155, 1725–1736.
- Galli, S.J. (1990). New insights into “the riddle of the mast cells”: microenvironmental regulation of mast cell development and phenotypic heterogeneity. *Lab. Invest.* 62, 5–33.
- Kitamura, Y. (1989). Heterogeneity of mast cells and phenotypic change between subpopulations. *Annu. Rev. Immunol.* 7, 59–76.
- Nakano, T., Sonoda, T., Hayashi, C., Yamatodani, A., Kanayama, Y., Yamamura, T., Asai, H., Yonezawa, T., Kitamura, Y., and Galli, S.J. (1985). Fate of bone marrow-derived cultured mast cells after intracutaneous, intraperitoneal and intravenous transfer into genetically mast cell deficient *W/W<sup>v</sup>* mice: evidence that cultured mast cells can give rise to both connective tissue-type and mucosal mast cells. *J. Exp. Med.* 162, 1025–1043.
- Ravetch, J.V., and Kinet, J.-P. (1991). Fc receptors. *Annu. Rev. Immunol.* 9, 457–492.
- Sylvestre, D.L., and Ravetch, J.V. (1994). Fc receptors initiate the Arthus reaction: redefining the inflammatory cascade. *Science* 265, 1095–1098.
- Sylvestre, D.L., Clynes, R., Ma, M., Warren, H., Carroll, M., and Ravetch, J.V. (1996). IgG mediated inflammatory responses develop normally in complement deficient mice. *J. Exp. Med.*, in press.
- Takai, T., Li, M., Sylvestre, D., Clynes R., and Ravetch, J.V. (1994). FcR  $\gamma$  chain deletion results in pleiotropic effector cell defects. *Cell* 76, 519–529.
- Walker, M., Harley, R., Maize, J., DeLustro, F., and LeRoy, E. (1985). Mast cells and their degranulation in the *Tsk* mouse model of scleroderma. *Proc. Soc. Exp. Biol. Med.* 180, 323–328.
- Wershil, B.K., Wang, Z.-S., Gordon, J.R., and Galli, S.J. (1991). Recruitment of neutrophils during IgE-dependent cutaneous late phase reactions in the mouse is mast-cell dependent: partial inhibition of the reaction with antiserum against tumor necrosis factor  $\alpha$ . *J. Clin. Invest.* 87, 446–453.
- Zhang, Y., Ramos, B.F., and Jakschik, B.A. (1991). Augmentation of reverse Arthus reaction by mast cells in mice. *J. Clin. Invest.* 88, 841–846.