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

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Summary

Antibody–antigen complexes are central to the inflammatory response and are implicated in the development of such diverse diseases as systemic lupus erythematosis, 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 FcγRIII 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 erythematosis, rheumatoid arthritis, immune vasculitis, and glomerulonephritis remain poorly understood. These macromolecular complexes are able to interact directly with effector cells through Fc receptors (FcγRs) 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.

FcγRs 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 FcγRI) or immune complexes (the low affinity receptors FcγRII and FcγRIII). These receptors share structurally related ligand-binding domains, but differ in their transmembrane and intracellular domains. In addition, both FcγRI and FcγRIII share with the IgE-binding receptor FcεRI their association with the γ subunit of the FcγR 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 γ subunit of the FcγR complex, and lacking functional FcγRI and FcγRIII as well as the high affinity receptor for IgE, FcεRI (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 FcγR engagement, resulting in cellular activation, chemotaxis, and infiltration of neutrophils. The FcγR-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 FcγRII and FcγRIII, or tissue macrophages, which express FcγRI, FcγRII, and FcγRIII (Ravetch and Kinet, 1991; Esposito-Farese et al., 1995). Previous studies have shown that the mast cell–deficient mouse line, W/W^v, exhibits an attenuated Arthus reaction, which is reconstituted by mast cell replacement. We therefore proposed that the mast cell FcγRIII 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^v with mast cells derived from wild-type or FcγR-deficient mice. As shown in Figure 1, bone marrow from γ −/− mice and littermate +/+ controls was harvested, cultured under conditions that result in homogeneous population of mast cells, and implanted intradermally into W/W^v 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 γ −/−, wild-type C57BL/6, and W/W^v 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. γ −/− mice (Figure 2A) show little detectable response, whereas +/+ control mice (Figure 2B) exhibit vigorous hemorrhage. The baseline W/W^v reaction (Figure 2C) is reduced by approximately 50%, as has been previously reported (Zhang et al., 1991). Reconstituting the W/W^v mice with mast cells derived from γ −/− animals (Figure 2D) does not result in any further augmentation of the Arthus response of the recipient W/W^v mice, while reconstitution with +/+ mast

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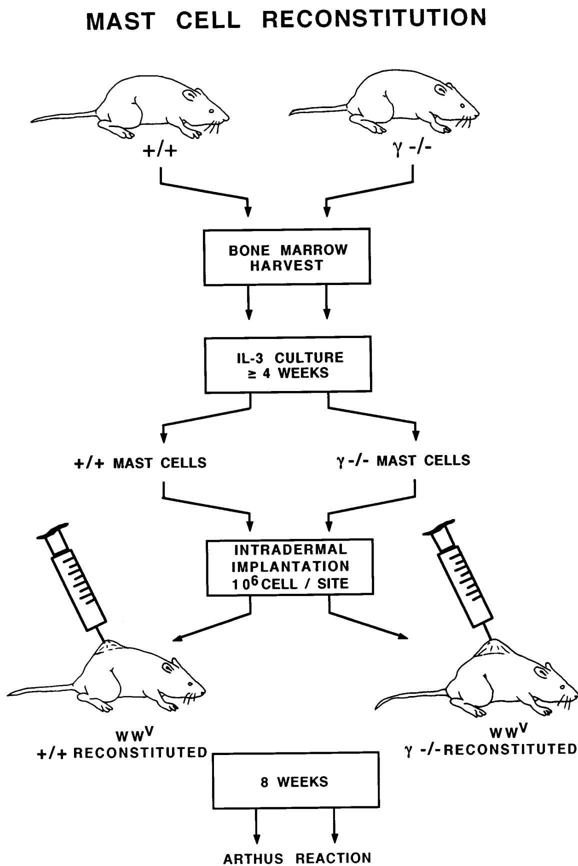

Figure 1. Protocol for Differential Reconstitution of Mast Cells

Bone marrow was harvested from γ $-/-$ 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 1×10^6 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}I-labeled human serum albumin for edema and the colorometric 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 mm^2 showed a density of 11 ± 3 for $+/+$ and 10 ± 3 for $-/-$.

The finding that mast cell FcγRIII 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 FcϵRI 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 FcγRs differ only in their α 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 γ $-/-$ mice. The partial response seen in the mast cell–deficient mouse, which is totally abolished in the γ $-/-$ mouse, argues for the involvement of another FcγR-bearing cell type that is required for reconstitution of the complete reaction. Tissue macrophages or Langerhans cells are FcγRIII 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 FcγR engaged. An understanding of the different pathways elicited by IgE and IgG when signaling through their respective mast cell FcγRs, as well as the cell types that contribute to the different reactions, should provide valuable insight into the process of inflammation.

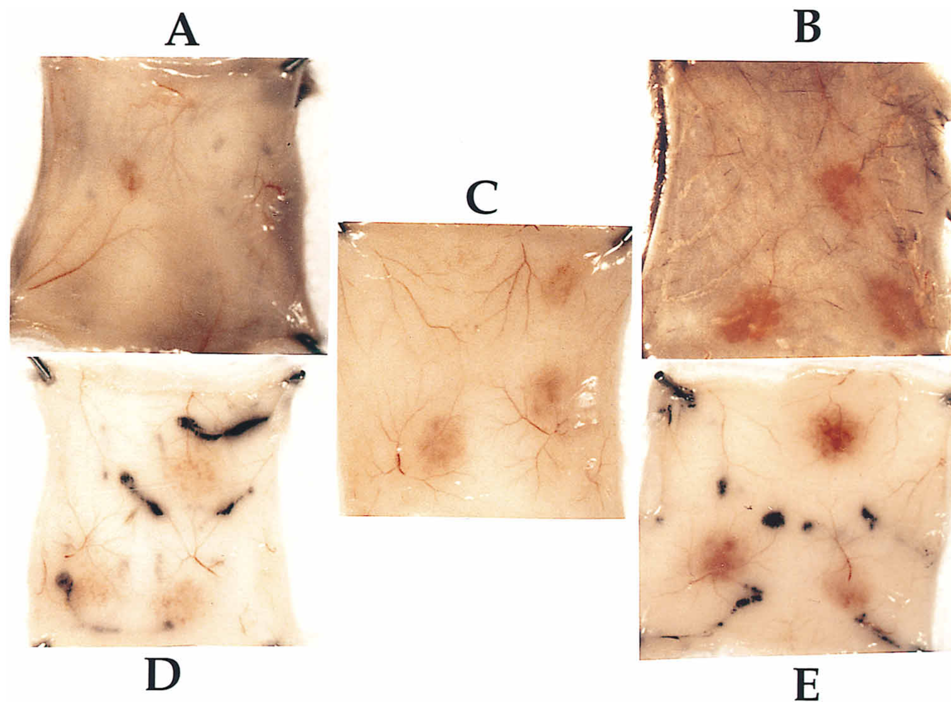

Figure 2. Hemorrhage from 8 hr Arthus Reaction

Inverted skin samples are shown from control γ −/− (A), +/+ (B), and unreconstituted W/W^v mice (C). W/W^v mice were reconstituted with either γ −/− 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 μg 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_2 asphyxiation, and bone marrow was flushed from femurs using sterile phosphate-buffered saline. Cells were resuspended at a density of 3×10^5 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 1×10^5. After 4–8 weeks of culture, the mast cells were washed and resuspended in phosphate-buffered saline at a density of 1×10^6 per 100 μl and immediately injected intracutaneously into the shaved dorsal skin of W/W^v 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 μg of either preimmune polyclonal rabbit IgG or polyclonal rabbit IgG against ovalbumin in a volume of 7.5 μl in a discrete spot, followed immediately by 20 mg/kg ovalbumin. Mice were sacrificed using CO_2 asphyxiation at either 2 or

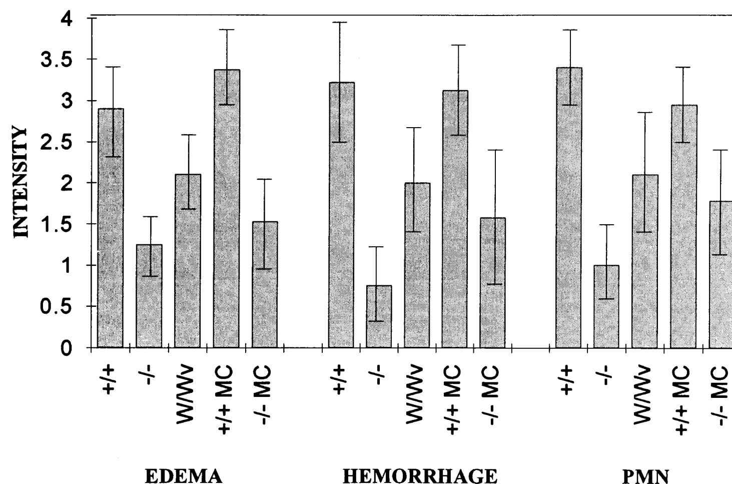

Figure 3. Microscopic Quantitation of the 8 hr Arthus Reaction

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 μg of antibody. Abbreviations are as follows: +/+, wild type; −/−, γ knockout; W/W^v, unreconstituted mast cell deficient; +/+ MC, W/W^v reconstituted with wild-type mast cells; −/− MC, W/W^v reconstituted with −/− mast cells. Results are based on at least 16 sections in each group; error bars are ± 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.

Acknowledgments

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