Experimental arthritis in rats induced by intra-articular injection of IgE aggregates: evidence for arthritogenic role of complexed IgE

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Abstract

An experimental arthritis model in the rat was used to study the arthritogenic potential of complexed IgE. IgE aggregates were produced in vitro by cross linking monoclonal rat IgE by dimethyl suberimidate and were injected into the knee joints. Animals which had not been injected and animals injected with phosphate buffered saline served as controls. The concentration of histamine in tissues, diffusion into the joint of bovine serum albumin labelled with iodine-125 injected intravenously, and the histology of the joints were studied. There was a significant decrease in the concentration of histamine in synovial tissue 8 and 24 hours after the injection of the IgE aggregates. A decreased number of stainable mast cells were found 8, 24, and 48 hours after exposure. A moderate hyperplasia of the synovial lining layer was also noted. These results provide further evidence for the arthritogenic potential of complexed IgE, especially in the initiation of arthritis through activation of mast cells.

It has been shown that immune complexes containing IgE are often found in patients with rheumatoid arthritis, especially those with severe arthritis with or without vasculitis.¹⁻⁴ We have also shown that these complexes are locally formed or trapped in synovial fluid⁵ and that complexes containing IgE isolated from synovial fluid can activate inflammatory cells.⁶

In this study, further evidence for the arthritogenic potential of these complexes was provided using an experimental arthritis model in rats.

Materials and methods

PREPARATION OF IgE AGGREGATES

Monoclonal rat IgE (IR2) was isolated and purified from ascites of rats bearing immunocytomas as described previously.⁷ Covalent cross linking was performed according to Segal *et al.*⁸ Briefly, IgE (26.5 mg/ml) was incubated for two hours at 30°C with a 30 fold molar excess (114 µg) of dimethyl suberimidate (Pierce Chemical, Rockford, IL, USA) in 0.2 mol/l TRIS hydrochloric acid, pH 8.5. Oligomers were precipitated with a final concentration of 15% polyethylene glycol 6000 and redissolved to 20 mg/ml in phosphate buffered saline (pH 7.40). The IgE aggregates were mainly dimeric, as shown by native polyacrylamide gel electrophoresis and by fractionation on a Sephacryl S-400 column (Pharmacia, Brussels, Belgium). The phosphate buffered

saline solution contained 50 µg/ml gentamicin and 30 U/ml nystatin (Gibco, Paisley, UK).

EXPERIMENTAL RATS AND INDUCTION OF ARTHRITIS

Outbred female Sprague-Dawley rats (Janssen Pharmaceutica, Beerse, Belgium) weighing approximately 250 g were anaesthetised with diethylether. Their hind legs were shaved and prepared using routine aseptic techniques. Two animals for each test received an intra-articular injection in two hind knees, obliquely through the patello-tibial ligament with a 25 gauge hypodermic needle. The following solutions were injected: 100 µg IgE aggregate diluted in 500 µl phosphate buffered saline solution (PBS), 1 µg IgE aggregate in 500 µl PBS, or 500 µl PBS only. The PBS solution contained 50 µg/ml gentamicin and 30 U/ml nystatin. Two additional rats for each test received no intra-articular injection.

DETERMINATION OF HISTAMINE CONCENTRATION IN TISSUES

The concentration of histamine in tissues was determined in the infrapatellar fat pads 1, 8, and 24 hours after intra-articular injection of the test solutions or in uninjected control rats. The rats were killed by ether overdose. The infrapatellar tissue was removed as described previously.⁹ The fat pads were immediately weighed, frozen, and stored in glass tubes at -20°C until assayed. After thawing, the tissues were frozen and thawed in 1 ml of distilled water twice more. The histamine concentration was determined in duplicate in 200 µl of supernatant with the Pharmacia histamine RIA 50 test. This commercial test uses histamine labelled with iodine-125 and a monoclonal mouse antibody to histamine. The results are expressed as nanograms of histamine per milligram of dry tissue weight.

DETERMINATION OF PERMEABILITY RESPONSE

The permeability of the synovial membrane was assessed using bovine serum albumin labelled with iodine-125 (NEN Research Products, Du Pont de Nemours, Brussels, Belgium), one and eight hours after intra-articular injection of the test solutions. Bovine serum albumin labelled with iodine-125 (111-185 kBq) and diluted in 500 µl phosphate buffered saline was injected into the tail vein 30 minutes before killing the rats. The infrapatellar fat pads were removed, weighed and the radioactivity determined in a

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gamma counter (Auto-gamma Scintillation Spectrometer, Packard Instruments, Downers Grove, IL, USA). The results were expressed as the ratio of the counts per milligram of dry tissue weight over the counts per 100 μ l of corresponding plasma, to correct for differences in the dose of bovine serum albumin labelled with iodine-125 injected.

COLLECTION AND PREPARATION OF JOINT TISSUES FOR HISTOPATHOLOGICAL EVALUATION

The rats were killed 8, 24 or 48 hours after intra-articular injection.

Light microscopy

For light microscopy the knee joints were collected at the same time by transverse section of the distal femur and the proximal tibia and fibula. The joints were trimmed of excess muscle and fixed in 10% neutral phosphate buffered formalin solution for one day. The joints were then decalcified for five days with a 10% formic acid-formalin solution. Subsequently, 3–4 mm gross sections were cut in a sagittal plane, dehydrated by graduated alcohols, embedded in paraffin and sectioned at 5 μ m. All histological sections were stained with haematoxylin and eosin, Masson's trichrome stain, and giemsa. Inflammatory changes were scored blindly. The following parameters were assessed: presence of polymorphonuclear cells with or without fibrin in the joint space; synovial lining layer proliferation; subsynovial infiltration with polymorphonuclear cells; oedema of the vascular wall with or without necrosis; number of mast cells, scored as the number of stainable mast cells for each of four fields along the synovial lining layer at a magnification of 125.

Immunofluorescence

The infrapatellar fat pad was examined to determine the immunofluorescence. Tissue was removed, immersed in Tissue-Tek (Miles Scientific, Naperville, IL, USA), frozen in liquid nitrogen and then stored for up to three weeks at -70°C until sectioned. Transverse

sections 8–10 μ m thick were cut at -20°C in a cryostat, picked up on glass slides, immersed in ice cold acetone for a few seconds and then air dried at room temperature. Before staining, the slides were stored at -70°C for up to one week.

For staining with fluorescein isothiocyanate conjugated antisera the tissue sections were warmed to room temperature, rinsed with phosphate buffered saline and incubated for 30 minutes at 37°C with 50 μ l of rabbit anti-rat fibrinogen (Janssen Biochimica, Beerse, Belgium), then diluted 1 to 50 in phosphate buffered saline containing 0.5% bovine serum albumin and 0.5% Tween 20 (PBT). After rinsing in phosphate buffered saline, the slides were incubated for 30 minutes at room temperature with 50 μ l of fluorescein labelled swine anti-rabbit antibodies (Dakopatts, Glostrup, Denmark), diluted 1 to 80 in PBT. Finally, the slides were rinsed with phosphate buffered saline, incubated for 20 minutes at room temperature with a stabiliser (Amersham, UK) and allowed to dry. Mountant (Amersham) and a coverslip were applied. For the detection of IgE, the tissue sections were incubated with 50 μ l of fluorescein labelled goat anti-rat IgE (Nordic Immunology, Tiburg, The Netherlands), diluted 1 to 50 in PBT. They were stabilised and covered as for the fibrin assay. The sections were examined with a Leitz Ortholux II fluorescence microscope.

STATISTICAL ANALYSIS

The results for the rats which had not been injected and rats injected with phosphate buffered saline were compared. As there was no clear difference between these groups, the results were pooled to one control group for the final analysis. The same procedure was followed for the groups injected with 100 and 1 μ g of IgE aggregate as the results for these two groups were also comparable. Statistical analysis was performed using non-parametric analysis of variance (Kruskal and Wallis) for numerical values and the χ^2 or Fisher's exact test for categorical data where appropriate. A p value of <0.05 was considered significant.

Results

HISTAMINE CONCENTRATION

Figure 1 shows that there was a significant difference in the histamine concentration between control rats (not injected) and injected with phosphate buffered saline) and the rats injected with IgE aggregates (100 or 1 μ g) at 8 and 24 hours after intra-articular injection ($p=0.02$), whereas there was no clear difference one hour after injection ($p=0.06$).

PERMEABILITY RESPONSE

No difference in the uptake of bovine serum albumin labelled with iodine-125 could be found between the knees of control rats and those injected with IgE aggregate ($p>0.20$).

HISTOLOGY

Mast cells

The most striking difference between the knees

Figure 1 Histamine concentration (nanograms per milligram of dry tissue weight; mean (SEM)) in infrapatellar fat pad tissue of control rats (C) and rats injected with 100 or 1 μ g of IgE aggregate (AlGE) at 1, 8, and 24 hours after injection. $n=4$ at each time point.

of control rats and rats injected with IgE aggregate was in the number of stainable mast cells. The mean (SEM) number of mast cells in control rats was 47 (9) compared with 28 (6) in the rats injected with IgE aggregate ($p<0.001$). In the group injected with IgE aggregate, the mast cell numbers were lowest 24 hours after the injection of IgE aggregate; the mean (SEM) numbers of mast cells at 8, 24 and 48 hours were 29 (5), 22 (3), and 31 (4), respectively.

Synovial lining layer proliferation

A synovial lining layer hyperplasia was noted in the knee joints of rats injected with IgE aggregate compared with the controls. The synovial lining layer was on average 3.6 (1.0) cell layers thick in the group injected with IgE aggregate compared with 1.6 (0.7) in the control group ($p<0.001$). Figure 2 shows that synovial lining layer hyperplasia could be observed eight hours after the injection of the IgE aggregates. No clear difference between the results obtained 8, 24 and 48 hours after injection was found.

Joint space

Figure 2 shows that a scarce infiltration of polymorphonuclear cells could be found in the joint space of rats injected with aggregate, especially eight hours after injection. However, rare polymorphonuclear cells were occasionally found in a few rats injected with phosphate buffered saline. Small fibrin deposits in the joint space were found in two thirds of the rats injected with IgE aggregate, but could not be found in the control group ($p<0.01$, rats injected with aggregate versus controls).

Subsynovium

A definite subsynovial infiltration was not consistently encountered in the rats injected with IgE aggregate nor in the control rats. Occasionally, polymorphonuclear cells could be

found in the subsynovium. The deposition of IgE could not be shown with the technique used. However, fibrin deposition could be detected by immunofluorescence in all the rats injected with IgE aggregate, whereas only one rat injected with phosphate buffered saline was positive ($p<0.001$ aggregate injected rats versus controls).

Vascular changes

Half of the rats injected with IgE aggregate showed mild oedema of the vascular wall. This was not seen in the control group ($p<0.05$, rats injected with IgE aggregate versus controls). Vascular necrosis was not found in any of the rats.

Discussion

An IgE dependent mast cell mediated arthritis model has been described previously.¹⁰ In this model mast cell degranulation and transient synovitis were induced by an intra-articular injection of antigen specific IgE and parenteral challenge with specific antigen. This model, as well as other reports,¹¹⁻¹³ suggests a possible role for IgE and mast cells in the initiation of arthritides. In addition several workers have focused on the possible role of mast cells in the pathophysiology of chronic inflammatory joint disorders in humans, especially in rheumatoid arthritis.¹⁴⁻¹⁸

Apart from monomeric IgE, there is a role for immune complexes containing IgE in rheumatoid arthritis, especially in severe arthritis and vasculitis.¹⁻⁴ It could be speculated that activation through IgE containing immune complexes of mast cells and other inflammatory cells such as monocytes and macrophages, possessing IgE type II receptors,¹⁹ could play a role in the

Figure 2 Photomicrograph of the sagittal knee joint section of a rat. (A) Eight hours after injection of IgE aggregates, showing synovial lining layer hyperplasia (I), inflammatory infiltrates (i) with rare polymorphonuclear leucocytes in the joint space (arrows) and synovium, and some fibrin deposition (arrowhead). (B) Eight hours after injection of phosphate buffered saline (control experiment). (Haematoxylin and eosin.)

inflammatory events in rheumatoid synovitis and rheumatoid vasculitis.

To study the arthritogenic potential of complexed IgE further, we developed an experimental animal model in rats, using the intra-articular injection of aggregated IgE. Former studies have already shown the inflammatory potential of IgE aggregates injected into the skin of guinea pigs and rats.^{20, 21} In this study it was shown that the intra-articular injection of IgE aggregates causes mast cell degranulation with a lowering of the concentration of histamine in tissues and a decreased number of stainable mast cells from eight hours after aggregate injection. In contrast with Malone and Metcalfe¹⁰ we found no statistically significant difference in the histamine content one hour after the injection of IgE aggregates and no marked increase in the permeability of the synovial membrane could be shown using intravenously injected bovine serum albumin labelled with iodine-125 as a marker. In our study, the relatively small number of rats and the wide range of values in the permeability study could explain this difference.

In a rabbit model, where immune complexes containing IgG were injected intra-articularly, a pronounced leucocyte infiltration into the joints was found eight hours after injection, especially when small immune complexes formed in an excess of antigen were used.^{22, 23} However, in our study mast cell triggering and moderate hyperplasia of the synovial lining layer were the main features and definite neutrophil or mononuclear cell infiltration was not consistently found. An explanation for the lack of neutrophil infiltration might be that aggregated IgE does not activate complement by the classical pathway and thus less chemotactic components (C3a, C5a, C567) are liberated.

In summary, this experimental arthritis model in rats provides further evidence for the arthritogenic potential of complexed IgE, especially in the initiation of arthritis by mast cell activation. In humans, where immune complexes containing IgE can be found intra-articularly and synovial mast cells are numerous, these events could be an initiating factor and/or an inflammatory amplification loop in the physiopathology of rheumatoid arthritis.

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