Histidine decarboxylase but not histamine receptor 1 or 2 deficiency protects from K/BxN serum-induced arthritis

Narendiran Rajasekaran\textsuperscript{1,6}, Samuel Solomon\textsuperscript{2}, Takeshi Watanabe\textsuperscript{3}, Hiroshi Ohtsu\textsuperscript{4}, Mieczyslaw Gajda\textsuperscript{5}, Rolf Bräuer\textsuperscript{5} and Harald Illges\textsuperscript{1}

\textsuperscript{1}Immunology and Cell Biology, University of Applied Sciences Bonn-Rhein-Sieg, von-Liebig-Strasse 20, D-53359 Rheinbach, Germany  
\textsuperscript{2}The Biomedical Research Centre, University of British Columbia, 2222 Health Sciences Mall, Vancouver, BCV6T 1Z3, Canada  
\textsuperscript{3}Department of Molecular Immunology, Medical Institute of Bioregulation, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka, Fukuoka 812-8582, Japan  
\textsuperscript{4}Graduate School of Engineering, Applied Quantum Medical Engineering, Tohoku University, Aza-Aoba 6-6-01, Aramaki, Aoba-ku, Sendai, 980-8579, Japan  
\textsuperscript{5}Institute of Pathology, University Hospital Jena, Ziegelmühlenweg 1, D-07740 Jena, Germany  
\textsuperscript{6}Present address: Department of Pediatrics, Stanford University, Stanford, CA 94305, USA

Keywords: histamine receptor, histidine decarboxylase, K/BxN mouse model

Abstract

Serum transfer from arthritic K/BxN mice into naive animals results in arthritis. Mast cells have been shown to be essential since mice lacking these cell type do not develop arthritis upon serum injection. Mast cell function depends on the release of granules filled with mediators such as histamine. Mice deficient in histidine decarboxylase (HDC\textsuperscript{−/−}) that do not produce histamine and mice deficient for histamine receptor 1 (H1R\textsuperscript{−/−}) or histamine receptor 2 (H2R\textsuperscript{−/−}) were injected with arthritogenic sera from the K/BxN mice, and the progression of arthritis was observed through the next 2 weeks. HDC\textsuperscript{−/−} mice that are histamine free developed a milder form of arthritis in comparison with the wild-type controls. In both receptor-deficient mice as well as in wild-type controls, the onset and severity of clinical arthritis and ankle thickening occurred during day 1 to 3. These results indicate that histamine is required but not indispensable for the development of serum-induced arthritis and histamine receptors other than those studied here may be involved.

Introduction

Crossing the TCR V\textsubscript{b}6 transgenic KRN mouse strain with the NOD/Lt mouse results in F1 mice (K/BxN) that develop arthritis spontaneously (1). In the arthritic mice, auto-antibodies are produced against the ubiquitous glycolytic enzyme glucose-6-phosphate isomerase dependent on both the TCR transgene and the NOD-derived IAg\textsuperscript{7} molecule (2). When sera or purified antibodies from the K/BxN mice are transferred to normal mice, a transient form of the arthritis develops. This serum transfer model has been used to identify various cellular and molecular components involved in the induction of inflammation. Neutrophils, mast cells and macrophages have been identified as important cellular factors required for disease development (3–5). Further, the arthritogenic antibodies act through the Fc\textsubscript{γ}RIII receptors and C5a and the alternate complement network is essential for the development of the disease (6, 7).

Mast cell-deficient mice were completely protected against K/BxN serum-induced arthritis (5) and mast cells in the synovium were found to degranulate within an hour of serum transfer into naive mice. Mast cell reconstitution of mast cell-deficient mice fully restored arthritis (8). Mast cell stabilization by compounds salbutamol or cromolyn within 24 h of sera injection prevented angiogenesis, pannus formation and joint destruction (8). Mast cells, thus, play an important role in the initiation of the disease. Mast cells secrete a variety of potential inflammatory mediators like histamine, leukotrienes, proteinases, heparin, vascular endothelial growth factor (VEGF) and various other cytokines.
including tumor necrosis factor-α (TNF-α) (9). The two important mediators histamine and TNF-α are capable of being released immediately after mast cell activation from preformed granules. Investigating the role histamine and its receptors during arthritis development will give us an insight into how mast cells mediate development of arthritis.

Histamine, a mediator of allergic reactions, is contained in the mast cell granules and is released during inflammation when mast cells are activated either by cross-linking the FceRI receptors or FcγRIII receptors (10, 11). Histamine acts as a vasodilator and chemoattractant through its receptors.

Four receptors of histamine, H1–H4, have been identified so far. These receptors differ in their tissue distribution and mediate different roles in inflammation depending on the location of the inflammation and the surrounding inflammatory milieu. The H1 receptor mediates endothelial cell and smooth muscle responses to histamine. Activation of the H1 receptor causes bronchoconstriction, vasodilatation and increased vascular permeability (12–14). H2 receptors on vascular smooth muscle cells also mediate vasodilatation (15).

To analyze whether histamine has a role in this disease model, we induced arthritis in histidine decarboxylase-deficient (HDC−/−) mice. Histidine decarboxylase is an essential enzyme in histamine biosynthesis and forms histamine from L-histidine and therefore, the HDC−/− mice do not make histamine. To further investigate if histamine's effect on arthritis is mediated through its receptors 1 and 2, we did K/BxN serum transfer in histamine receptor 1-deficient (H1R−/−) and histamine receptor 2-deficient (H2R−/−) mice.
Methods

Experimental animals

The KRN transgenic mice were a kind gift from D. Mathis and C. Benoist (IGMBC, Strasbourg, France) (1). C57BL/6 (B6) mice and NOD/Lt mice were obtained from our animal facility and were maintained under pathogen-free conditions. HDC−/− mice were obtained from Prof. Hiroshi Ohtsu (16). H1R−/− and H2R−/− mice were obtained from Prof. Takeshi Watanabe (17, 18). The KRN and K/BxN strains were bred as described earlier (7).

Induction of arthritis by K/BxN serum transfer and arthritis assessment

Arthritis was induced in the recipient mice by an intraperitoneal injection of 200 µl of the K/BxN sera (7). Increase in ankle thickening was measured using a micrometer (Hann and Kolb, model no. 33185). Ankle thickness, the summed average of the thickness of the four limbs per mouse, was expressed in millimeters. Clinical index score, independent on the number of ankles affected, was measured in a scale varying from 0 to 4. Histology was performed and evaluated in a blinded manner as described (19).

Statistical analysis

The data were expressed as the means ± SEMs. Statistical analysis was performed with SPSS for Windows version 10.0 (SPSS, Chicago, IL, USA). Data were analyzed with the Mann-Whitney U-test. For each test, P values ≤0.05 were considered significant.

Histochemistry

Mice were sacrificed by cervical dislocation. Ankle joints were removed, skinned and prepared by fixing for 24 h in 4% phosphate-buffered formaldehyde. Fixed joints were decalcified by treatment with Osteosoft (Merck KGaA, Darmstadt, Germany) for 1 week. Samples were then washed with PBS, dehydrated with a series of ethanol washes (50% ethanol, followed by 70% ethanol) and embedded in paraffin. Sections of tissue 4 µm thick were cut and stained with hematoxylin and eosin (H & E). Four sections per joint were examined and scored in a blinded manner for the extent of inflammation and joint destruction. The extent of joint inflammation was defined by synovial hyperplasia and degree of infiltration of the synovial membrane by granulocytes and scored as follows: 0, no infiltration; 1, mild infiltration; 2, moderate infiltration and 3, severe infiltration.
the damage to cartilage and bone structures (structural bone defects and cartilage cell necrosis) was evaluated on a scale of 0–3, where 0—no damage, 1—mild destruction, 2—medium damage of bony matrix and 3—severe damage of bone (extensive area of destruction, deep invasive destructions of bone).

Results
To elucidate the role of histamine in serum-induced arthritis, we injected 200 μl K/BxN sera intraperitoneally into HDC−/− mice and C57Bl/6 wild-type controls. Onset and progression of arthritis was observed through the next 2 weeks determining ankle thickness by caliper measurement and a clinical determining the numbers of affected joints. The onset of clinical arthritis and ankle thickening occurred around 24–48 h in both HDC−/− and control mice, but HDC−/− mice, which are not able to produce histamine, developed a milder form of arthritis when compared with the wild-type mice (Fig. 1A and B). Inflammation was assessed histologically from ankle sections of mice sacrificed on different days during the course of arthritis. Histological examination of the HDC−/− mice showed a reduced severity of arthritis (synovial inflammation, pannus formation; Fig. 1C) when compared with the wild-type controls (Fig. 1D). Statistical analysis showed significant differences in both ankle thickness (days 4, 6, 8 and 10) and clinical index (days 2, 6, 8 and 10). Together, this shows that absence of histamine ameliorates serum-induced arthritis but does not protect the mice from arthritis.

To determine the strength of the rheumatic disease in our experimental mice, we examined four sections per joint and scored in a blinded manner for the extent of inflammation (synovial hyperplasia, cellular infiltration of synovial tissue) and joint destruction (pannus formation, cartilage and bone degradation). The results of this analysis (Fig. 2) show that both inflammation and joint destruction are significantly reduced in the absence of histidine decarboxylase (HDC) in the deficient mice.

In order to elucidate which receptors are involved in mediating the histamine effects on arthritis, K/BxN sera were injected into H1R−/− or H2R−/− mice and C57Bl/6 wild-type controls. Mice were experimentally treated and analyzed in the same way as described above for the HDC-deficient animals. There were no detectable differences in the onset of ankle thickening (Figs 3A and 4A), clinical arthritis (Figs 3B and 4B) and histological severity of arthritis (Fig. 3C and D and Fig. 4C and D) between both types of histamine receptor-deficient and control mice. Our results indicate that both examined histamine receptors have no essential role in this serum-induced arthritis model.
Histidine decarboxylase deficiency protects from K/BxN serum-induced arthritis

**Discussion**

From the above experiments, we can conclude that histamine is essential for arthritis development but not indispensable. Alternatively, mediators other than histamine that promote mast cell activity might be important. In this context, it was already demonstrated that VEGF, which promotes vasodilatation and angiogenesis, is essential for arthritis development in the K/BxN model (20). Another important candidate is TNF-α. Like histamine, TNF-α is released by mast cells as a preformed mediator but is also synthesized later for a sustained release (21). TNF-α is also capable of mediating vasodilatation (22, 23). Mast cell-secreted TNF-α has been shown to play an important role in immune complex-mediated diseases. The K/BxN serum-induced arthritis is basically an immune complex-mediated type III hypersensitivity reaction. In a model of immune complex-induced inflammation of the peritoneal cavity, TNF-α secreted by mast cells was shown to augment neutrophil emigration (24). In a mouse model of rheumatoid factor-mediated skin vasculitis, mast cells were essential for triggering vasculitis and mast cells mediated their action through FcγRI/II receptor and TNF-α (25). Further investigations are being carried out to find if TNF-α is also an important mediator of mast cell effects in this model of arthritis.

Histamine mediates its action through a variety of four different receptors (H1–H4) (26–28). These receptors differ in their tissue distribution (26, 29). They mediate different roles in inflammation depending on the location of the inflammation and the surrounding inflammatory milieu (27). Though H1 and H2 receptors do not have a role in K/BxN serum-induced arthritis, histamine may still mediate its effects through the other two receptors H3 and H4. The expression of histamine receptor 4 in the synovial cells from rheumatoid arthritis patients has been recently shown (30, 31). Further, in the absence of one of the receptors in the knockout mice, its functions may be compensated by the other receptors. Another possibility is that absence of both the H1 and H2 receptors may be required to show a pronounced effect on the disease.

In conclusion, our study shows that histamine is required for the development of K/BxN serum-induced arthritis. However, the role of histamine receptors is unclear and the involvement of other receptors of histamine like H3 and H4 need to be investigated.

**Funding**

EU grant (MRTN-CT-2004-005693); Deutsche Forschungsgemeinschaft (BR 1372/9).

**Acknowledgements**

The authors appreciate the excellent technical support of Cornelia Hutlich.

Conflict of Interest: The authors declare that they have no conflict of interest.

**Abbreviations**

HDC histidine decarboxylase
HDC−/− histidine decarboxylase-deficient
H1R−/− histamine receptor 1-deficient
H2R−/− histamine receptor 2-deficient
H & E hematoxylin and eosin
TNF-α tumor necrosis factor-α
VEGF vascular endothelial growth factor

**References**


