Antigen induced arthritis in beige (Chediak-Higashi) mice

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Abstract
Mice with the beige mutation, which are known to be deficient for leucocyte elastase and cathepsin G, were used to investigate the role of neutral proteases in a model for antigen induced arthritis. Surprisingly, it was shown that in this model of arthritis, using methylated bovine serum albumin as an antigen, C57/black/6 'beige' mice (deficient for leucocyte neutral proteases) developed a more severe form of arthritis than the control mice ('black' mice), resulting in a higher degree of tissue damage. The incidence and degree of bone apposition and destruction of articular cartilage at day 21 after induction of arthritis were significantly higher in the beige mice. These findings could not be ascribed to differences in the cellular immune response to methylated bovine serum albumin. Autoradiographic detection of radiolabelled methylated bovine serum albumin suggested that more antigen is retained in the joints of beige mice than in black mice, which might account for the sustained arthritis and the concomitant tissue damage. These findings do not support the contention that leucocyte elastase and cathepsin G contribute to the pathogenesis of joint destruction in this model.

Recently, a mutant mouse has been described which is deficient for polymorphonuclear leucocyte elastase and cathepsin G.¹ This mutation, the so-called 'beige' gene, represents a putative model for the Chediak-Higashi syndrome in humans, with which it has several features in common, such as the giant lysosomes and the neutral protease deficiency. The beige mouse is therefore a suitable model for investigating the role of neutral proteases in various inflammatory conditions. We have recently described the effect of experimental arthritis, using the model of zymosan induced arthritis, in beige mice.² It was found that although in vitro beige polymorphonuclear leucocytes caused less cartilage degradation, the tissue damage resulting from inflammation was not significantly different from that in the black mice. It was therefore concluded that polymorphonuclear leucocyte elastase and cathepsin G were not the main mediators of cartilage breakdown in this model. Because in a glomerulonephritis model based on immunological mechanisms we found significant differences between beige and black mice³ we sought to compare the effect of an immunologically mediated experimental arthritis on beige and black mice. We therefore chose the model of allergic arthritis which uses methylated bovine serum albumin as an antigen, as described before.⁴ We have previously shown that part of the inflammatory reaction in this model, at least in the acute phase, is dependent on hydrogen peroxide generated by activated inflammatory cells.⁵ As in the zymosan induced arthritis model, the findings in this study do not support a role for neutral proteases.

Materials and methods

ANIMALS
Male, normal (black) C57Bl/6 and beige C57Bl/6 (bg/bg) mice were obtained from Harlan Olac, UK. The animals (aged 10–16 weeks) were fed a standard diet and tap water freely.

CHEMICALS
Methylated bovine serum albumin was obtained from Sigma Chemicals Co, USA. Methoxy-succinyl-alanyl-alanyl-prolyl-valyl-aminomethyl-coumarin (MAAPV-AMC) was obtained from Bachem, Bubendorf, Switzerland.¹²¹ was obtained from Amersham, Bucks, UK.

ELASTASE ASSAY
Elastase was used as a marker enzyme to compare beige and black polymorphonuclear leucocytes. This enzyme was assayed as previously described using the fluorogenic substrate MAAPV-AMC.⁶

INDUCTION OF ARTHRITIS
Joint inflammation was induced as described previously.⁴ Briefly, mice were immunised with methylated bovine serum albumin in complete Freund's adjuvant, and arthritis was induced by injection of 60 µg of the antigen in the right knee joint three weeks after the primary immunisation. This type of inflammation is dependent on T lymphocytes and is characterised by an infiltrate rich in polymorphonuclear leucocytes in the acute phase (first week) and a mononuclear infiltrate in the chronic phase (up to four weeks). The immune status of the animals (delayed type hypersensitivity against methylated bovine serum albumin) was measured by skin testing. Antigen (10 µg) was injected in the right ear and the swelling after 24 and 48 hours was measured with a caliper.⁵

MEASUREMENT OF ARTHRITIS
Joint inflammation was measured at days 2, 5, 7, and 21 by the technetium-99m method.⁷
Briefly, the accumulation of $^{99m}$Tc is measured in the right (R) and left (L) control knee joint and expressed as a ratio (R/L). This ratio is taken as a measure for joint swelling. Ratios above 1.1 were considered to indicate inflammation of the right knee joint.

**RETENTION OF RADIOLABELLED METHYLATED BOVINE SERUM ALBUMIN**

Methylated bovine serum albumin was radio-labelled by the chloramine T method. To investigate the effect of arthritis on the clearance of antigen from the inflamed joint in the beige and normal mice radio-labelled antigen (37 kBq) was injected, and the retained antigen was monitored by external gammacounting or by autoradiography. Previous studies have indicated that this method does follow the fate of the injected antigen and that the measured radiolabel actually represents immunoreactive methylated bovine serum albumin.

**HISTOLOGY AND AUTORADIOGRAPHY**

Mouse knee joints (six to eight in each group at each timepoint) were processed for histology and autoradiography. A histological examination was made on days 2, 5, and 21. Arthritis was scored on serial sections (haematoxylin and eosin staining). Infiltration, cartilage damage, and bone apposition were scored semi-quantitatively. Antigen retention was visualised by autoradiography of $^{125}$I labelled methylated bovine serum albumin, and was scored on a four point scale. All histological examinations were performed 'blindly' by two observers.

**Results**

**MEASUREMENT OF EXPERIMENTAL ARTHRITIS IN BEIGE AND BLACK MICE**

Black and beige mice were immunised with methylated bovine serum albumin in complete Freund's adjuvant. No differences in delayed type hypersensitivity (skin testing) were found between the two strains (data not shown). Arthritis was induced by the intra-articular injection of 60 μg of antigen in saline. Table 1 shows the time course of arthritis as assessed by $^{99m}$Tc uptake, which is taken as a measure of joint oedema. At days 7 and 21 $^{99m}$Tc uptake was significantly higher in the beige (neutral protease deficient) mice than in the black mice, indicating that vascular effects of inflammation were prominent in the beige mice. For all experiments the elastase content of polymorphonuclear leucocytes from both strains was measured as a routine check of the neutral proteinase deficiency. For the groups of mice used in these studies the elastase content of beige mice was always found to be less than 10% of that of the black mice (data not shown).

**HISTOLOGY OF ARTHRITIS**

Histological examination of the joints showed that the amount of cellular infiltrate in the beige mice was higher throughout the inflammatory response, though it only reached statistical significance at day 5 (table 2). Figure 1 shows that the infiltrate and exudate were predominantly

| Table 2: Histological examination of arthritis in beige and black mice. Values are means (SD) |
|---|---|---|---|
| Day | Mice | Infiltrate | Cartilage damage | Bone apposition |
| 2 | beige | 1.2 (0.4) | ND | ND |
| 2 | black | 1.3 (0.5) | ND | ND |
| 5 | beige | 3.0 (0.1) | ND | ND |
| 5 | black | 1.9 (0.5)* | ND | ND |
| 21 | beige | 1.4 (0.5) | 2.6 (0.8) | 2.4 (0.8) |
| 21 | black | 1.1 (0.7) | 1.3 (1.0)* | 1.0 (1.0)* |

Seven to eight mice a group were injected with 60 μg of methylated bovine serum albumin at day 0. At several intervals animals were killed for histological examination. The amount of cellular infiltrate in the synovium and periostium was scored semi-quantitatively on serial sections at days 2, 5, and 21. Cartilage damage and bone apposition was only present in the chronic phase of arthritis (day 21). The histological grading is given on a four point scale in proportion to severity.

Figure 1: Arthritic knee joint of C57B1/6 beige mice at day 2 after induction of inflammation. Note the exudate in the joint space and the cellular infiltration of the synovium (mainly polymorphonuclear leucocytes). P = patella; F = femur; S = synovium. (Haematoxylin and eosin staining.) Bar = 100 μm.
nantely composed of polymorphonuclear leukocytes in the acute phase. At day 21, when inflammation was waning, a significant difference in irreversible end stage joint destruction was seen between the two strains (Table 2). The beige mice then showed considerably more cartilage destruction and apposition of newly formed bone than the black mice. Figures 2 and 3 show the extent of joint deformation resulting from this type of inflammation in the beige mice: total destruction of articular cartilage and enormous humps of newly formed bone.

**Antigen retention in beige and black mice**

The data described above are contrary to what would be expected if neutral proteases had a role in joint destruction during inflammation. Surprisingly, cartilage destruction was even more severe in the beige mice. A possible explanation for this phenomenon might be that polymorphonuclear leukocyte neutral proteases actually assist with the elimination of the inflammatory stimulus—for example, by degradation of immune complexes, and thereby contribute to attenuation of the inflammatory response rather than promoting it. To investigate this possibility we measured the clearance of antigen from the inflamed joint by following the amount of radiolabelled antigen during the course of arthritis, using external gamma-counting. It was found that the black mice eliminated the antigen factor faster than the beige mice, though the difference never reached significance (data not shown). As external gamma-counting of radiolabelled antigen is a rather crude method for detecting the antigen (as it measures antigen in the entire knee joint, including some of the periarticular tissues) we also studied the fate of the injected antigen in situ, using autoradiography at day 8 after injection of radiolabelled antigen. Previous studies have shown that radiolabelled material detected by autoradiography can be equated with immunoreactive antigen. When the amount of
retained antigen was assessed semiquantiative
ly with a four point scale on serial sections (as
shown in fig 4) a small, but significant dif-
ference was found between beige and black mice.
Black mice: mean (SD) 1·9 (0·3) (n=9) and
beige mice: 2·6 (0·7) (n=6), p<0·05, Wilcoxon
rank sum test. This indicates that at least at day
8 more antigen was present in the joints of beige
mice than in those of black mice.

Discussion
Beige mice, deficient for polymorphonuclear
leucocyte elastase and cathepsin G, and their
normal C57 black/6 counterparts were used in
the methylated bovine serum albumin induced
arthrits model. The hypothesis that the de-
iciency of polymorphonuclear leucocyte neutral
proteases would decrease tissue damage or chron-
icity of the inflammatory response was proved
wrong. It was shown that beige mice developed
a more serious and more chronic lesion than
black mice, resulting in a more pronounced
irreversible damage to articular tissues.

Neutral proteases derived from polymor-
phonuclear leucocytes have been strongly impli-
cated in the degradation of articular cartilage
in various arthritic conditions. In septic arthritis,
gout, and in active rheumatoid arthritis massive
infiltration and exudation of polymorphonuclear
leucocytes lead to the release of lysosomal
enzymes in the joint space. Several in vitro
studies have shown that elastase and cathepsin
G can degrade articular cartilage proteo-
glycans,11 12 and elastase was shown to be
present in rheumatoid cartilage.13 Few in vivo
studies are available, however, that unequi-
vocally show the part played by polymorpho-
nuclear leucocyte neutral proteases in tissue
damage. Recently, we have studied two models
of inflammation in beige and black mice. It was
found that in experimental arthritis induced
with zymosan there was no significant difference
in inflammation or tissue damage between the
two strains.2 In a model for experimental
glomerulonephritis, however, we showed a
significant difference in proteinuria between
beige and black mice, suggesting that poly-
morphonuclear leucocyte neutral proteases
caused damage to the glomerular basal mem-
brane.5 In this paper we have investigated the
antigen induced arthritis model using beige and
black mice. This arthritis model is dependent
on T lymphocytes, though polymorphonuclear
leucocytes are probably the effector cells in the
acute phase as we have previously shown that
hydrogen peroxide contributes to the acute
vascular effects in this model.5 Surprisingly, it
was found that the inflammatory response and
the amount of tissue damage in the beige mice
were even higher than in the black mice. In the
chronic phase beige mice showed considerably
more bone apposition and destruction of articular
cartilage. The only difference between the
two strains (as known so far) is the amount of
elastase and cathepsin G. No differences in
collagenase or acid protease content could be
shown.1 We have also shown that there are no
significant differences in the production of
superoxide.2 As the neutral proteases might,
alternatively, assist in the removal of the
inflammatory stimulus (proteinolysis of antigen or
immune complexes) we studied the clearance
rate of labelled antigen from the joint. Our
studies suggest a slight difference in antigen
clearance between the two strains. The possible
role of elastase and cathepsin G in the degrada-
tion of antigenic material is currently being
investigated. In this model neutral proteases
might eventually represent an off-switch mecha-
nism for inflammation rather than a
potentiating mechanism.

The tissue damage found in the beige mice
might also be explained by the action of other
neutral proteases such as elastase or cathepsin G
operating in joint inflammation, such as oxygen
metabolites2 14 and cytokines.15 When these
mediators are produced in excess the absence
of neutral proteases would not make much differ-
ence to the resulting tissue damage. The relative
contribution of the various mediators is at present
not known and is a subject of further
investigation.

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