Avridine-induced arthritis in rats; a T cell-dependent chronic disease influenced both by MHC genes and by non-MHC genes

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SUMMARY

Avridine is a potent synthetic adjuvant that can induce arthritis in most rat strains. The clinical appearance and histopathology of avridine-induced arthritis show great similarity to other arthritis models such as collagen-induced arthritis. In LEW and DA rats the avridine-induced arthritis is severe and long lasting. To investigate a possible genetic influence on the disease we compared LEW, DA and E3 rats, which are of different genetic origins, for their ability to develop arthritis after injection of a low dose of avridine (1·5 mg/rat). The E3 rat was shown to be resistant, whereas all of the DA rats developed arthritis. Recombinant inbred strains derived from DA and E3 parentals varied in susceptibility to avridine. Only strains sharing $RT1^{av1}$ with DA developed arthritis, indicating a role for the MHC genes. The MHC association was further analysed in a series of Lewis congenic strains using the 1·5 mg avridine dose. All strains developed arthritis. LEW.1C and LEW.1W developed only acute arthritis, whereas LEW.1A, LEW, LEW.1D, LEW.1N and LEW.1F developed chronic arthritis. In particular, the LEW.1F rats developed a chronic severe arthritis of high incidence. The chronic arthritis showed an active, erosive joint inflammation several months after induction. Nude rats are resistant to avridine-induced arthritis, indicating a T cell dependence of the disease which supports the importance of MHC. However, non-MHC genes are also crucial to arthritis development. Recombinants between DA and E3, sharing $RT1^{av1}$ with DA, showed either a lower incidence or a lower severity of disease than the DA rats. The E3 rat and the recombinants with $RT1^{u}$ were completely resistant, whereas LEW.1W, also $RT1^{u}$, were highly susceptible.

Keywords adjuvant arthritis   autoimmunity   MHC   congenic rats

INTRODUCTION

There are several different animal models for rheumatoid arthritis (RA) currently used. The collagen-induced arthritis (CIA) is based on autoimmune recognition of a joint-derived autoantigen (reviewed in [1]). This disease is clearly immunological in nature and dependent on both genes within the MHC, called RT1 in the rat, and those outside the MHC. There is a strict MHC association of this disease to $RT1^{a}$ as the most permissive haplotype, with other haplotypes varying in permissiveness depending on the gene background [2]. Adjuvant arthritis can be induced with various adjuvants, such as mycobacteria tuberculosum in mineral oil [3], avridine in mineral oil [4], muramyl dipeptide [5], pristane [6] or Freund's mineral oil only [7]. The model most extensively used is the mycobacteria tuberculosum-induced adjuvant arthritis, a severe but non-chronic disease [8]. This disease is clearly T cell-dependent, since nude rats are resistant and antibody

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treatment against the T cell receptor prevents development of disease [9]. The susceptibility is polygenetically associated with as yet unknown non-MHC genes, as exemplified with the susceptibility of the LEW rat in comparison with the resistant F344 rat [10, 11]. There are, however, reports that MHC may also influence disease susceptibility, indicating that the $RT1^{av1}$ haplotype of the DA rat is permissive for disease susceptibility [11]. The pathogenesis of adjuvant arthritis induced with mycobacteria tuberculosum is, however, not clarified. The classical view is that the disease induction is triggered by adjuvants [12], whereas later experiments suggest that autoreactive T cells cross-reacting with mycobacteria heat shock proteins mediate the disease [13]. In this context, it is of interest that severe arthritis can also be induced with other adjuvants such as avridine, pristane and mineral oil. These oils and synthetic compounds contain no immunogenic material to be presented to T cells or to be a target for an antigen-specific immune response [4]. Therefore we believe it is important to characterize these models in order to understand the endogenous mechanisms leading to arthritis. In this report we study

the avridine-induced arthritis (AvIA). This is an easily repro-
ducible disease which appears at predicted onset days and with
predicted severity in contrast to many other models such as
CIA and the mycobacteria tuberculosum-induced arthritis. We
show here that the AvIA is a chronic, T cell-dependent disease
which is influenced by both MHC and non-MHC genes.

MATERIALS AND METHODS

Rats

Specific pathogen-free rats obtained from ZFV (Zentralinstitut
für Versuchstierzucht, Hannover, Germany) were bred and
kept at the Biomedical Centre in Uppsala. The rats were kept
in a climate-controlled environment with 12 h light/dark cycles,
housed in polystyrene cages containing wood shavings and fed
standard rodent chow and water *ad libitum*. All experiments
were performed on age- and sex-matched rats at an age of 8–14
weeks. The rats were found to be free from common pathogens
including Sendai virus, Hantaan virus, coronavirus, reovirus,
cytomegalovirus and *Mycoplasma pulmonis*.

Induction and evaluation of arthritis

N,N-dioctadecyl-N′,N′-bis(2-hydroxyethyl) propanediamine
(= CP20 961 = avridine) was generously provided by Dr
W.W. Hoffman (Pfizer Inc., Groton, CT). Arthritis was
induced in the rats by a subcutaneous injection at the base of
the tail of 150 μl avridine solubilized in Freund's incomplete
adjuvant (FIA; Difco, Detroit, MI) at a concentration of 0, 10,
25 or 50 mg/ml. Arthritis development was monitored daily by
a macroscopic scoring system for the four limbs ranging from 0
to 4 (1 = swelling and/or redness of one phalangeal joint;
2 = two phalangeal joints or of one larger joint involved;
3 = more than two joints involved; and 4 = severe arthritis in
the entire paw).

Histomorphological and immunohistochemical analysis of joints

Joints were fixed in 4% paraformaldehyde, rapidly decalcified,
embedded in paraffin, sectioned and stained with haematoxylin
and eosin. For the immunohistochemichal analysis the joints
were decalcified for 6–8 weeks with EDTA [14]. Cryosections
were stained with MoAbs using the avidin–biotin–peroxidase
complex technique [15]. MoAbs to αβ T cell receptor, R73 [16];
CD4, W3/25 [17]; CD8, OX8 [18]; MHC class II, OX6 [19]; IL-2
receptor (CD25), ART18 [20]; complement receptor CR3
(CD11b), OX42 [21] and CD43 on neutrophils, some macro-
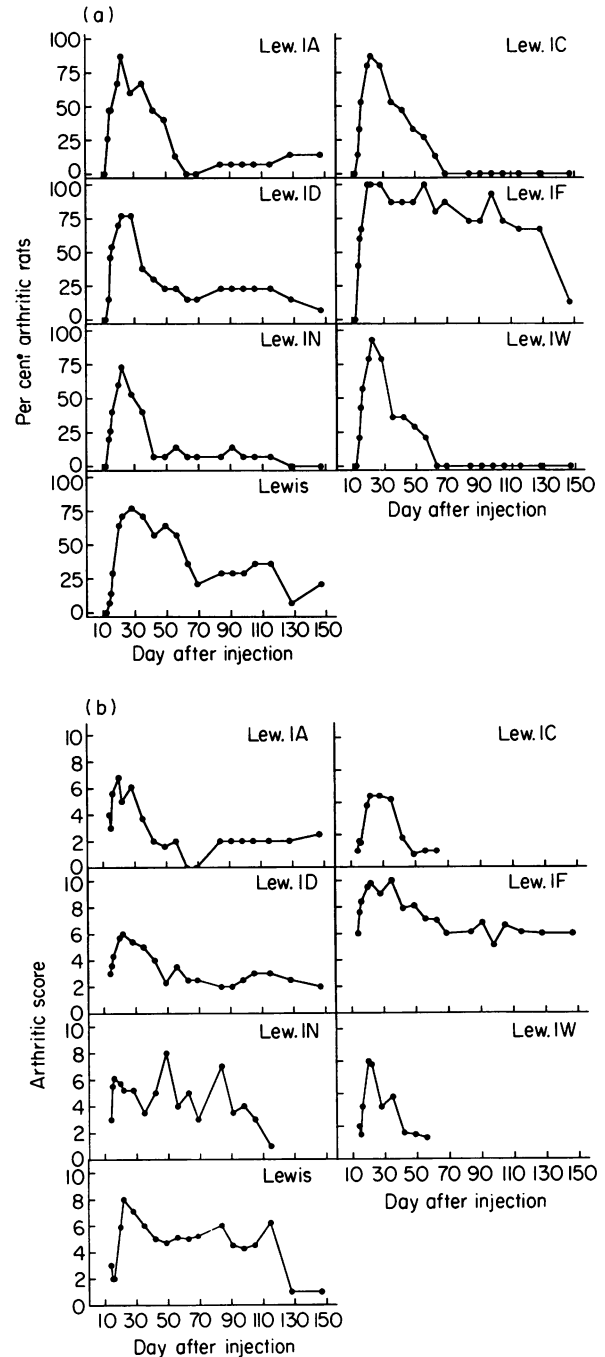phages and T cells, W3/13 [17] were used.

Fig. 1. Development of arthritis in the different MHC congenic strains
is shown as arthritis frequency (a) and arthritis severity (b). The
arthritic score is calculated as the mean score of arthritic animals
only. This is a summary of two experiments with similar results. The
number of rats of each strain is presented in Table 2.

Table 1. Titration of the avridine dose for induction of arthritis in LEW
rats

Avridine dose/rat (mg)	Number of rats	Incidence (%)	Mean day of onset	Mean maximal severity
0 (only FIA)	6	0	–	–
0·75	6	0	–	–
1·5	14	86	15 ± 3	11 ± 4
3·75	6	100	13 ± 2	15 ± 0
7·5	10	90	11 ± 1	10 ± 6

RESULTS

Susceptibility to AvIA in DA, LEW and E3 rats

We have earlier observed that both LEW and DA rats develop
severe arthritis with early onset and 90–100% frequency after
injection of 7·5 mg avridine/rat. The LEW rats were used to
titrate the dose of avridine (Table 1). We found that 1·5 mg
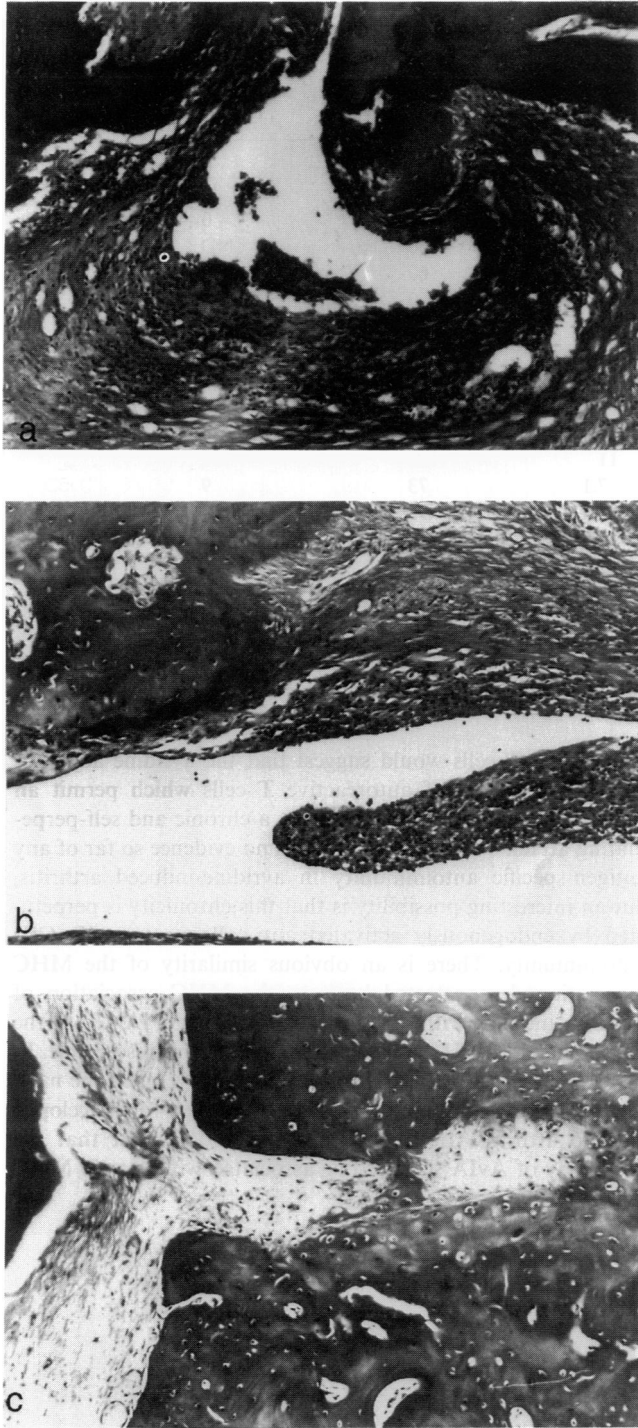avridine per rat was the lowest arthritogenic dose, whereas

Fig. 2. Haematoxylin–eosin-stained formalin-fixed sections from different rats taken 178 days after avridine injection. (a) A hind paw from a LEW.1F rat with chronic arthritis with severe local erosions and inflammatory cells. (b) A front paw from a LEW.1A rat with recurrence of arthritis as shown by a new focal inflammatory infiltrate. (c) A hind paw from a LEW.1W rat which has recovered from acute arthritis showing an ankylotic joint with fibrotic synovial tissue but with no sign of active arthritis.

higher doses induced arthritis with earlier onset and higher severity. Comparison of three different rat strains (DA, LEW and E3) with unrelated gene backgrounds for arthritis susceptibility using the lower (1·5 mg/rat) dose showed a variable susceptibility ranging from the resistant E3 rat to the highly susceptible DA rat (Table 2). E3 rats injected with the higher dose (7·5 mg avridine/rat) did develop arthritis, but with lower incidence and severity. To initiate a genetic analysis we compared inbred recombinant strains with mixed DA and E3 genes (Table 2). These rat strains developed arthritis with a variable frequency and severity. Interestingly, only strains carrying the RT1^{avl} haplotype were susceptible. This prompted us to analyse more closely the role of MHC.

MHC congenic strains

A series of LEW RT1 congenic strains, listed in Table 2, was analysed for arthritis development after injection of 1·5 mg avridine/rat. The results, shown in Fig. 1a,b and in Table 2, show that all strains began to develop arthritis within 2 weeks after injection of avridine, reaching a maximal incidence around day 28. The subsequent disease course did vary, however. LEW.1F rats developed a severe arthritis with a chronic disease course. The ongoing arthritis severely damaged the paws, and after several months the activity of disease was not easily determined by clinical scoring. Histopathology sections taken from paws 6 months after injection showed severe inflammation with active erosions of bone and cartilage (Fig. 2a). In contrast, other strains, LEW.1W and LEW.1C, developed only acute arthritis, and the paws completely recovered from clinical signs of arthritis after 2–3 months. Histopathological sections taken after 6 months showed scars after erosions but only fibrotic tissue and no active inflammation (Fig. 2c). In other strains, LEW.1A, LEW.1D and LEW.1N, a few rats developed a chronic course of active arthritis. Interestingly, in a few LEW and LEW.1A rats a marked reappearance of active arthritis was seen several months after avridine injection. A chronic erosive disease was seen in the histopathological analyses of these rats as well (Fig. 2b). Neither of the RT1 congenic strains developed any clinical signs of arthritis after injection with FIA alone within the 22 days of observation.

AvIA is a T-cell dependent disease

Avridine is a non-immunogenic adjuvant and it is not known if the arthritis involves immune cells or if it develops in response to a non-specific triggering by the adjuvant. We therefore wanted to analyse if T cells were involved. We compared nude thymus deficient WAG rats with their nu/+ littermates (Table 2). This experiment showed that normal thymus is required for the disease. The T cell infiltration and MHC class II expression were investigated in paws from Lewis rats injected with 1·5 mg avridine/rat (Table 3). We found an early infiltration of CD4$^+$ T lymphocytes and significant MHC class II expression in these paws just before the onset of clinical arthritis.

DISCUSSION

The findings described in this study show that avridine-induced arthritis is a T cell-dependent, chronic disease which is genetically associated with genes both within and outside the MHC. It is important to emphasize that the disease is induced with a

Table 2. Development of arthritis in different rat strains

Strain	RT1	n	Mean day of onset	Mean max. severity	Acute arthritis (%)	Chronic arthritis (%)
DA	avl	16	14 ± 1	11	100	0
E3	u	11	–	–	0	0
E3*	u	6	21 ± 5	3	50	0
DXEA	avl	4	12 ± 0	2	100	ND
DXEB	avl	5	21 ± 4	5	60	ND
DXEC	u	5	–	–	0	–
DXER	u	5	–	–	0	–
LEW	l	14	19 ± 4	8	86	34
LEW.1A	a	15	16 ± 4	6.7	80	7
LEW.1C	c	15	17 ± 3	7.1	87	0
LEW.1D	d	13	16 ± 2	6.2	85	28
LEW.1F	f	15	15 ± 4	11	100	100
LEW.1N	n	15	17 ± 3	7.1	73	9
LEW.1W	u	14	17 ± 3	8.4	93	0
WAG nu/nu*	u	5	–	–	0	ND
WAG nu/+*	u	5	15 ± 1	7.2	100	ND

The rats were injected with 1·5 mg avridine (*7·5 mg). The DA rats and the Lewis congenic strains were observed for 150 days and the E3, DXEA, DXEB, DXEC and DXER for 40 days. The mean maximum severity is calculated on arthritic rats only, as the mean of each individual rat's maximal score. ND, No data.

synthetic adjuvant with no known antigenic capacity of its own [4]. Thus, no foreign immunogenic material has been introduced. The mechanism by which avridine induces arthritis is as obscure as in other adjuvant arthritis models such as the mycobacteria tuberculosum-induced disease, the muramyl dipeptide-induced arthritis, the oil-induced arthritis or the pristane-induced arthritis. However, as with the other adjuvant-induced arthritides [5, 6, 9], the avridine-induced arthritis is T cell-dependent, since nude rats are resistant. This finding is in accordance with the previous demonstration of a successful transfer of avridine-induced arthritis by concanavalin A (Con A)-activated lymphocytes [22]. The MHC class II expression in the joints and the significant but limited T cell infiltration is seen also in other autoimmune models such as CIA and adjuvant arthritis induced with mycobacteria [23–25]. A criti-

cal role for T cells would suggest that the avridine adjuvant triggers activation of autoreactive T cells which permit an initial acute arthritis and eventually a chronic and self-perpetuating attack on the joints. There is no evidence so far of any antigen-specific autoimmunity in avridine-induced arthritis, but an interesting possibility is that this chronicity is perpetuated by endogenously activated anti-collagen type II (CII) autoimmunity. There is an obvious similarity of the MHC association demonstrated here, to the MHC association of the CIA induced with homologous rat CII in FIA [2]. In the CIA model the RT1^a and RT1^f haplotypes are susceptible. In the present experiments the LEW.1F rats developed the most severe arthritis. In the other strains only a few rats developed chronic arthritis. In this context it is of interest that the chronicity of AvIA seems to be associated with certain MHC

Table 3. Immunohistochemical analyses of paws from LEW rats injected with 1·5 mg avridine/rat

Staining of cell surface marker (MoAb)	12 days after avridine injection	21 days after avridine injection	21 days after control injection
CD11 (OX42)	+ + +	+ + +	+ +
CD43 (W3/13)	+ +	+	–
CD4 (W3/25)	+ +	+ +	+
CD8 (OX8)	+	+	–
TCR αβ (R73)	+	+	–
TCRVβ8.2 (R78)	–	+	ND
TCRVβ8.5 (B73)	+	–	–
TCRVβ10 (G101)	+	–	–
CD25 (ART18)	+	–	–
MHC class II (OX6)	+ +	+ +	+

– = 0%; + < 0·5%; + + = 0·5–5%; + + + = 5–20%. Day 12 shows the mean of five rats and day 21 the mean of three rats. Control rats represent one rat injected with Freund's incomplete adjuvant (FIA) and two rats injected with ovalbumin emulsified in FIA.

haplotypes. MHC class I and class II molecules are coded from the MHC, and are the restriction elements for $CD8^+$ and $CD4^+$ $\alpha\beta$ T cells. However, a number of other polymorphic genes may also be of importance, such as tumour necrosis factor genes [26], peptide transporter genes [27], complement genes [28] or heat shock protein genes [26]. It was also apparent from the presented results that non-MHC genes influence the susceptibility to AvIA, which is also the case in other autoimmune arthritis models. The availability of inbred and recombinant strains with variable susceptibility to disease is a valuable tool for analysing these putatively disease-promoting genes.

It is apparent that genetic analysis will be of importance also for widening the understanding of the pathogenesis of RA in humans. There is a rather low, but clearly significant, genetic association of RA [29]. So far only MHC genes have been identified, albeit without a deeper knowledge of their biological role or interacting autoantigens [30]. Apparently other genes are also important in exerting a complex and polygenic influence on disease susceptibility. We think well characterized animal models will be of help in sorting out these genes, and in helping to understand the biological roles of different arthritis-promoting genes.

ACKNOWLEDGMENTS

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