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Arthritis induced in rats with non-immunogenic adjuvants as models for rheumatoid arthritis

Summary:

Rat models are useful for studies of the pathogenesis of rheumatoid arthritis (RA) since rats are extraordinarily sensitive to induction of arthritis with adjuvants. Injection of not only the classical complete Freund's adjuvant but also mineral oil without mycobacteria and pure adjuvants such as pristane and squalene, induce severe arthritis in many rat strains. Models like pristane-induced arthritis in rats are optimal models for RA since they fulfill the RA criteria including a chronic relapsing disease course. Arthritogenic adjuvants like pristane, avridine, squalene and mineral oil are not immunogenic since they do not contain major histocompatibility complex (MHC) binding peptides. Nevertheless, the diseases are MHC-associated and dependent on the activation of $\alpha\beta$ TCR (T-cell receptor)-expressing T cells. However, it has not been possible to link the immune response to joint antigens or other endogenous components although immunization with various cartilage proteins induce arthritis but with different pathogeneses. To unravel the mechanisms behind adjuvant-induced arthritis, a disease-oriented genetic approach is optimal. Several loci that control onset of arthritis, severity and chronicity of the disease have been identified in genetic crosses and most of these have been confirmed in congenic strains. In addition, many of these loci are found in other autoimmune models in the rat as well as associated with arthritis in mice and humans.

Introduction

Rheumatoid arthritis (RA) is a clinically heterogeneous disease defined by a number of clinical criteria. It seems also to be genetically heterogeneous. RA is most likely a syndrome comprising several diseases, which are caused by different pathogenic mechanisms. It is therefore reasonable to expect that no particular RA model will mirror all aspects of the human disease, but the various animal models can be used to delineate different pathways leading to arthritis.

This review focuses on a category of RA models in which joint inflammation is triggered by a single intradermal injection of an immunostimulatory agent (adjuvant). Adjuvant arthritis demonstrates that joint-inflammation can be triggered by non-specific stimulation of the immune system. This mode of arthritis induction has so far received little attention,

although it is likely to give important clues to the pathogenesis of RA.

Several of the discussed adjuvant arthritis models fulfill the clinical definition of RA (Table 1) and display at least some of the following characteristics:

- The disease is tissue-specific and affects primarily diarthrodial, cartilaginous peripheral joints, although typical systemic manifestations may also occur.
- Bone and cartilage are eroded by the inflammatory tissue.
- No persisting infectious agents are found that could explain inflammation.
- The disease is chronic.
- The disease severity and chronicity are associated with certain MHC class II haplotypes.
- The disease does not develop spontaneously, but is induced by immunostimulatory agents in susceptible individuals that harbor a set of disease-associated alleles.

Arthritis can also be induced in the rat after immunization with cartilage-derived proteins like collagen II (CII), collagen XI (CXI) and cartilage oligomeric matrix protein (COMP) which fulfill many of these criteria as well. However, certain of these models are more optimal models for RA in that the rats are more prone to develop chronic arthritis. These models are autologous CII- and CXI-induced arthritis in the cartilage protein group as well as pristane- and avidine-induced arthritis in the adjuvant arthritis group. Nevertheless, it is clear that the adjuvant arthritis involves a different type of pathogenesis than that induced by cartilage protein models.

While cartilage protein-induced models are established in several species (mice, rats and apes), reproducible adjuvant models appear to be restricted to rats (1–4). In only one case has an adjuvant has been shown to induce arthritis in the mouse (5). This occurs after repeated pristane injections in the peritoneum but the disease is not joint specific and does not resemble pristane-induced arthritis in rats (6). The fact that adjuvant arthritis cannot easily be investigated in the mouse has disadvantages, including lack of embryonic stem cell-based technology, less developed genetic and immunological tools, and higher costs for breeding and maintenance. Rats do, however, offer some advantages as compared to mice. For example, they are more prone to develop a chronic relapsing disease course, the inflammatory responses are better described such as the acute phase response, and the larger size of the animal facilitates surgery and examinations. Furthermore, the fact that rats are susceptible to both cartilage protein-induced and adjuvant-induced arthritis permits parallel

Table 1. Characteristics of RA and some selected rat models in the DA rat^a

	RA	MIA	OIA	SIA	AvIA	PIA	Autologous CIA	Autologous CXIA	COMP/IA
Bone and cartilage erosions	+++	+++ (114)	-/+ (30, 31)	++ (68)	+++ (78)	+++ (6)	+++ (2)	+++ (39)	+ (34)
Enthesopathy and new bone formation	+/-	+++ (114)	-	-(68)	++ (78)	++	+++ (2)	+++ (39)	- (34)
T-cell infiltration in the joints	+++ (115)	++ (17)	+ (31)	++ (68)	++ (78)	++ (6)	+	++ (39)	nr
Circulating COMP	+++ (116)	nr	nr	nr	nr	+++ (112)	+++ (117)	nr	nr
Symmetric involvement of peripheral joints	+	-	+ (33)	+ (68)	nr	+ (110)	+	+	nr
Chronicity (sustained or relapsing joint inflammation and erosion)	+++	- (114)	- (31, 118)	-(68)	-/++++ (78)	+++ (6)	+++ (36, 38)	+++ (39)	- (34)
Rheumatoid factors	+++	-	nr	nr	nr	++	nr	nr	nr
Immune response to cartilage	++ (CII) (119)	-	nr	nr	-	-	++ (37, 38)	++ (39)	+ (34)
MHC association ^b	DR4, DR1 shared epitope alleles (120)	av1>I (59, 90)	av1>n (33) av1>h (40)	av1>h (68)	≡a>u.c.d.n (78)	≡a.u.c.d.n (6, 92)	a>u>f ≡c.d.n (38)	≡u>a, l, n, c (39)	u>l>f n>c.d (34)
Gender preponderance	Females	Females	Females	No/Females (68)	Females (121)	Females (6)	Females (121)	Males (39)	nr

^a The selected models are described in the main text. The various traits are quantitatively graded using a scale from +++ (pronounced), ++ (clearly present), + (barely significant), to - (not present). A slash (/) indicates variability, nr=not reported. References are lacking if the description is based on unpublished observations made in the authors' laboratories or are common textbook knowledge.

^b The data are based on MHC congenic strains on both DA and LEW backgrounds as well as genetic segregation experiments.

studies of the two disease types, often in the same strains. This is important since adjuvants are also used in cartilage protein-induced arthritis to break tolerance to the injected auto-antigen. Cartilage proteins have, in fact, been shown to be therapeutic in adjuvant arthritis through nasal vaccination (7).

This review focuses on the advantages of the rat adjuvant arthritis models with special reference to the understanding of the chronicity of the disease and to studies of adjuvant-induced pathways leading to arthritis through a genetic approach.

History of animal models

The formulation of commercial immunological adjuvants was the result of intense research in the early 20th century. The aim was to enhance immunity towards tuberculosis, and to achieve high-titered antisera against bacteria. In 1947, Jules Freund introduced a mixture of mineral oils, heat-killed mycobacteria and emulsifying agent, designated complete Freund's adjuvant (CFA) (8, 9). This concoction proved to be an efficient enhancer of both cell-mediated and humoral immune responses towards the antigens with which it was emulsified. Repetitive immunizations were sometimes used, but were severely detrimental to health, since mycobacteria cause formation of persistent foci of inflammation, which are often necrotic (granuloma). Mycobacteria were therefore often omitted, in which case the adjuvant was called incomplete Freund's adjuvant (IFA). Most of the pioneering adjuvant studies were carried out in rabbits and guinea pigs, but once CFA was formulated it became widely used in many species. In 1955, Lipton & Freund demonstrated in rats that tolerance to central nervous system tissue could effectively be broken by immunization of the tissue together with CFA (9, 10). Since then, mixtures of adjuvant and auto-antigen(s) have been routinely used to induce a wide variety of experimental autoimmune diseases, mainly in rats and mice.

Contemporary with Lipton and Freund, Stoerck reported in 1954 that joint lesions developed in rats after immunization with CFA and rat spleen tissue (11). Stoerck suspected the spleen tissue to be arthritogenic, but Pearson demonstrated that CFA and not the spleen component was responsible for development of joint inflammation and established the adjuvant arthritis model in 1956 (1, 12). A thorough characterization of this CFA-induced arthritis (hereafter denoted mycobacteria-induced arthritis, MIA) showed that the disease was not joint-specific. It was associated with widespread inflammatory infiltrates and granuloma formation in

many organs, for example in the spleen, liver, bone marrow, meninges, skin and eyes. MIA is a severe but self-limiting disease and the rats recover within a few months. These early descriptive findings evoked questions about the causative mechanisms. One hypothesis was that the joint inflammation develops as a consequence of immunity towards microbial antigens spreading to the joints (13). However, this idea lost support when it was demonstrated that arthritis can be transferred from diseased to healthy irradiated recipients by cells (14), later shown to be T lymphocytes (15–17), expressing CD4 (18), and $\alpha\beta$ TCR (T-cell receptor) (19). To explain the role of T cells, several investigators favored another idea, namely that mycobacterial antigens elicit a pathological T-cell immunity that includes cross-reactions with joint antigens (20, 21). This way of breaking tolerance is termed molecular mimicry (22) and it is suggested to be the cause of many autoimmune diseases, including RA (23). One line of study suggested that the evolutionarily conserved 65 kD heat shock protein (HSP) was the immune target in MIA, and also in RA (24, 25). However, this protein was unable to trigger arthritis (26). While the focus was on HSP, the search for a minimal arthritogenic component of mycobacteria had already led to the identification, in 1977, of a peptidoglycan cell wall fragment which is non-immunogenic but has adjuvant properties, i.e. muramyl dipeptide (MDP) (27, 28). It was also reported that mycobacterial components are not even necessary to trigger arthritis, since a synthetic non-immunogenic adjuvant called avridine could substitute for mycobacteria (29). Thus, both MDP and avridine can induce arthritis when injected together with incomplete Freund's adjuvant (IFA). Furthermore, we later unexpectedly discovered that IFA (i.e. mineral oil) alone, induced arthritis in DA rats (30, 31). This oil-induced arthritis developed also in germ-free DA rats, demonstrating that microorganisms are not involved in the pathogenesis and that responses to heat shock proteins did not occur in the germfree rats (32). However, most other rat strains, including the LEW (Lewis) strain, which is highly susceptible to MIA, was resistant to mineral oil-induced arthritis (OIA) (31, 33, 34). Taken together, these data infer that the mycobacterial component and the oil cause arthritis by separate mechanisms. Although it has not yet been documented that mycobacteria can induce arthritis without mineral oil, aqueous suspensions of cell walls from other bacteria, for example streptococci (streptococcal cell wall-induced arthritis, SCWIA), are arthritogenic (35). Therefore, we will in this review discuss "adjuvant arthritis" as being caused by 'pure' adjuvants (like mineral oil, pristane, avridine or squalene) without involvement of bacterial cell walls or

other components known to bind to immune adaptive or innate receptors.

Another turn was taken in 1977 when it was discovered that immunization with cartilage-derived type II collagen (CII)-induced arthritis in Wistar rats (2). The CII was emulsified with CFA or IFA but it could be shown that denatured CII in CFA or IFA failed to induce arthritis in the same strain demonstrating that collagen-induced arthritis (CIA) was different from the earlier described MIA. The CIA model offered a major advantage since the inducing immunogen could be characterized and it did also highlight the importance of cartilage as a target tissue in arthritis.

Arthritis induced by immunogenic cartilage antigens

Several different cartilage proteins have been shown to induce arthritis in rats. The requirements are that an autoimmune response is triggered and that the protein is located in the target tissue. The immune response requires an MHC class II molecule that can select and bind a peptide from the cartilage protein used for immunization and it also requires the capacity of T cells to respond to this peptide. However, the sensitivity and disease pathways seem to differ between different cartilage protein-induced models which may be dependent on the nature of the autoantigen, i.e. its location and interaction with the immune system may affect the tolerance state of autoreactive T and B cells. So far, three different cartilage-derived proteins have been shown to induce arthritis in rats; type II collagen (CII), type XI collagen (CXI) and cartilage oligomeric matrix protein (COMP).

Collagen-induced arthritis (CIA)

The CIA model in the rat is in many aspects similar to the corresponding mouse disease, which is perhaps the most commonly used model for RA today. However, the rat model offers several advantages compared with the mouse model in that the rat is more susceptible to induction with autologous CII, which results in a more pronounced chronic relapsing disease. As in RA, females are more susceptible in the rat model, whereas there is a male preponderance in the mouse model. In addition, it allows direct comparisons with the adjuvant type of arthritis models, which is interesting as these diseases appear to be caused by different pathogenic mechanisms. A major difference is that pathways involving B cells are engaged in CIA to a greater extent than in adjuvant arthritis models.

Intradermal injection, in DA rats, with native autologous rat collagen, emulsified in IFA, leads to a severe, erosive poly-

arthritis suddenly developing 2–3 weeks after immunisation followed by a subsequent chronic relapsing phase (36–38). The disease is genetically controlled both by MHC genes and other genes (37, 39, 40). Interestingly, the MHC association is limited to the RT1a haplotype with RT1u and RT1f being intermediate responders. This is different from the induction of arthritis with heterologous CII, or with CII, contaminated with pepsin, which show a very broad MHC-association pattern (39, 41). This is most likely related to the selected activation of non-self reactive T cells, specific for peptides derived from heterologous CII or pepsin that differ from the self rat CII (42). Another hallmark of the CIA model is the strong B-cell response specific for triple helical epitopes (38, 43). These B cells are autoreactive and produce arthritogenic antibodies (44, 45). Thus the disease involves activation of both T and B cells that are antigen-specific and autoreactive. An unresolved issue, however, is the relative importance of T and B cells during the effector phase of the disease. The development of arthritis is obviously a very complex process that can occur through different inflammatory mechanisms and that varies between strains and species. It is therefore difficult to interpret the large number of diverging effects on CIA, which have been obtained using mice ablated for various genes of putative importance for arthritis. It is clear, however, that a significant portion of the inflammatory attack on the joints is mediated by pathogenic antibodies (44–48). A role for autoreactive T cells in CIA, induced by immunization with autologous CII, is suggested by the finding that the arthritis is reduced by treatment with antibodies to the $\alpha\beta$ TCR at the onset of the disease, i.e. after the establishment of the antibody response (49). Passive transfer of the disease by CII-reactive T cells, however, is not as effective as in other autoimmune models such as experimental allergic encephalomyelitis (50) or as in adjuvant type of arthritis models (16). A cooperation between T and B cells in the effector phase has been suggested by combined transfer experiments (51, 52). It is essential to note that the identification of autoreactive CII-specific T cells has not yet been achieved and it is likely that these cells are tolerized but still arthritogenic as has been suggested in the mouse CIA model (53). Histopathologic analysis of the inflamed joints supports the notion that the arthritis is mediated by different effector arms and develops through different stages (45, 54–56). Antibodies produced by CII-specific B cells bind to the cartilage surface and may through binding of C1q form immune complexes which trigger Fc-receptors on synovial cells leading to infiltration of neutrophilic granulocytes and severe edematous arthritis. T cells and T-cell derived cytokines promote differentiation and

activation of macrophages, osteoclasts and fibroblasts and the development of a highly vascularized pannus tissue, leading to an aggressive subchondral erosive process. A healing response with new bone and cartilage formation is often seen a few weeks after the onset of arthritis, leading to restructuring and ankylosis of the joints.

It is clear that the disease is very complex and varies depending on animal strain, depending on whether the induction is made with heterologous or autologous protein, and it is influenced by environmental factors. In addition, it will occur in stages, which depend on different pathogenic mechanisms. The outstanding problem will be to identify the critical molecular interactions that lead to the disease. This question can be answered only by a disease-oriented approach as was initiated in the rat CIA model by Wilder and co-workers by performing comparative studies of susceptible and resistant strains to identify the critical genetic polymorphisms, which are associated with the disease (57, 58). A large number of gene regions, which contain susceptibility genes, have been identified in several crosses, using the DA strain as the susceptible counterpart and comparing this with other resistant strains such as F344, PVG and BN (57–62). These studies show that the disease is indeed complex and polygenic. In each cross a selected set of genes seems to provide susceptibility and it is likely that different genes control specific events during the development of disease. The results are reviewed in detail elsewhere in this issue and will here only be discussed in conjunction with results obtained from genetic analyses of the adjuvant arthritis models.

Collagen type XI-induced arthritis (C^{XI}IA)

CXI is, as CII, mainly located in cartilage but it is less abundant. It is intermingled in the collagen fibers together with CII. Like CII it is a long triple helical molecule, containing three α chains. One of the chains (α_3) is shared with CII whereas the α_1 and α_2 chains are unique. Induction of arthritis with CXI shows striking similarities with the CII-induced disease in that a chronic relapsing disease occurs after immunization with autologous CXI (39), whereas a more self-limited arthritis occurs after immunization with heterologous CXI (63, 64). These similarities cannot be explained by the shared CXI α_3 /CII α_1 chain since the immune response to CXI seems to be directed towards the CXI α_1 and CXI α_2 chains rather than the CXI α_3 chain both at the T-cell and the B-cell levels (39, 64). Thus, the antibody response is not cross-reactive with CII but is, as after CII immunization, directed towards the conformational triple helical structure. Consequently, the MHC association pattern differs between

CIA and C^{XI}IA. Development of C^{XI}IA induced with autologous rat CXI is strongly associated with the RT1f haplotype, whereas other haplotypes, including RT1a, are low responders. In contrast, RT1a is a high responder MHC class II haplotype for response to CII and development of CIA. There are also other differences between the two models. The C^{XI}IA is more aggressive in its chronic disease course and histopathologic analysis identifies germinal center-like clusters of B cells in the joints. Surprisingly, males are more susceptible to C^{XI}IA. It is therefore likely that the genetic control of CIA and C^{XI}IA differs with regard to other genes than those encoded from the MHC.

Cartilage oligomeric matrix protein-induced arthritis (COMPIA)

COMP is a cartilage-specific molecule that induces an acute and self-limited arthritis in several rat strains (34). It is associated with the RT1u MHC haplotype and surprisingly it is readily inducible in the E3 strain that is resistant to CIA as well as all adjuvant arthritis models tested so far. This suggests that COMPIA is dependent on unique disease pathways. The COMP protein is released from cartilage both during physiologic cartilage growth and during an arthritic erosive process. Circulating fragments of COMP can therefore be used for monitoring of the arthritis process. The ease with which the arthritis is induced by immunization with COMP is therefore surprising and immune epitopes, which are absent from these circulating fragments, are likely to be targeted.

Arthritis induced by non-immunogenic adjuvants

The observation that arthritis can be induced by adjuvant, which lacks bacterial cell wall components or any other components that contain peptides, which is a requirement for binding to classical MHC molecules, made it interesting to investigate the adjuvant type of arthritis models. A challenge is to delineate how non-peptide-specific stimulation of the rat immune system can lead to a disease with extensive similarities to an auto-immune disease like RA. For example, pristane- and avidine-induced arthritis are chronic, joint-specific, regulated by T cells and by MHC genes and fulfill the clinical criteria for RA. Yet, there is no evidence for auto-immune reactions.

Structure of arthritogenic adjuvants

As is the case for experimental auto-immune disease where inducing antigens often have been subjected to detailed characterization, researchers in the adjuvant arthritis field have

tried to identify and molecularly define arthritogenic adjuvants (Table 2). This line of research has led to the identification of a large number of arthritogenic agents, including defined microbial structures (27, 28, 65) and synthetic adjuvant molecules derived from both animals and plants (6, 29, 65). In addition, several types of arthritogenic oils have been identified based on the fact that IFA is not a pure oil, but contains 85% mineral oils (Bayol F) and 15% emulsifier (Arlacel A). Surprisingly, this led to the discovery that the endogenous cholesterol precursor squalene is arthritogenic (65), i.e. it causes an 'auto-adjuvant'-induced arthritis. Furthermore, arthritogenic pristane is also present in normal tissue, including thymus and peripheral lymphoid organs, since it is a component of chlorophyll and is normally ingested by all mammals including laboratory rats and humans (66, 67). Collectively, these data tell us that joint inflammation can be triggered by a variety of structurally unrelated adjuvants of different origin.

It is clear that several of the described adjuvants can elicit antibody responses under particular circumstances, for example when used as a hapten. They are, however, generally reported to be non-immunogenic. It is also conceivable that lipid adjuvants can bind to CD1 and be recognized by CD1-restricted T cells, but there is no evidence to support this hypothesis. Finally, the fact that there exist a large number of different arthritogenic adjuvants makes it highly unlikely that they are contaminated with peptide antigens. Moreover, the question of protein contamination was specifically addressed in squalene-induced arthritis (68). It was demonstrated that a putative peptide contaminant would be given to rats at levels far below the amounts needed for the induction of cartilage protein-induced autoimmune arthritis in rats and in mice. Taken together, the results suggest that the arthritogenic adjuvants cause disease based on their capacity to non-specifically stimulate the immune system.

Several of the adjuvants described above are suitable for mechanistic studies, and some have given rise to useful models for studies of arthritis. Here we will focus our discussion on arthritis induced by non-immunogenic adjuvants: incomplete Freund's adjuvant (i.e. OIA), avridine (AvIA), squalene (SIA) and pristane (PIA).

Clinical characteristics of commonly used adjuvant arthritis models

OIA (30, 31), AvIA (29), SIA (68) and PIA (6) share many features but differ with regard to severity and chronicity of the disease. They are induced with adjuvant compounds that lack apparent immunogenic capacity, i.e. no specific immune

Table 2. Examples of structurally different arthritogenic adjuvants of varying origin

Type of structure	Molecule	Origin
Peptidoglycans	Muramyl dipeptide (MDP)*	<i>M. tuberculosis</i>
Polysaccharides	β -glucan	<i>S. cerevisiae</i>
Glycolipids	Lipopolysaccharide*	<i>E. coli</i>
	TDM (trehalose dimycolate)*	<i>M. tuberculosis</i>
Lipids	C ₃₀ H ₅₀ (squalene)	eukaryotic
	C ₁₆ H ₃₄ (hexadecane)	
	C ₁₇ H ₃₆ (heptadecane)	
	C ₃₈ H ₈₀ NBr (DDA)*	
	C ₄₃ H ₅₂ N ₂ O ₂ (avridine)*	
	C ₁₉ H ₄₀ (pristane)	Plants

* LPS, TDM, DDA, avridine and MDP are arthritogenic only together with IFA. The table is adapted from (65). This article demonstrates the arthritogenicity of TDM, LPS, β -glucan, squalene, and DDA. This is confirmed for squalene (68), β -glucan (122) and DDA (123). Arthritis induced with MDP (muramyl dipeptide), avridine, and pristane are established models. PIA and AvIA are chronic (6, 78).

responses are elicited towards them after intradermal injection at the base of the tail. Macroscopic arthritis appears in peripheral joints after a delay of at least 1 or 2 weeks, with a similar distribution as in RA. Inflamed joints contain several inflammatory cell types, including T cells. Erosion of bone and cartilage occurs in all models, but most prominently in chronic arthritis that develops with certain combinations of adjuvants and strains, for example PIA in DA rats. Other joints may occasionally be involved, but no inflammatory manifestations in other tissues have been reported so far. In most cases, joint inflammation cannot be re-induced by a second adjuvant injection. This "vaccination effect" occurs both in animals that have recovered from arthritis and in rats that did not develop arthritis after the first challenge.

Dissemination of adjuvants and early responses to adjuvant provocation

For induction, a small volume of adjuvant (100–200 μ l) is injected intradermally in the skin. The administration route is of critical importance. For example, we have observed that pristane and β -glucan induce arthritis only if injected intradermally or subcutaneously, not if injected intraperitoneally or given perorally ((6) and unpublished observations, R. Bockermann, J. C. Lorentzen, R. Holmdahl). Within a few minutes (<15 min) after injection the arthritogenic effect of the injected adjuvants disappears from the injection site and spreads in the body. Using ¹⁴C-labeled hexadecane in DA rats and whole body autoradiography, it was demonstrated that the injected arthritogenic oil spread rapidly and, surprisingly,

with high selectivity for lymph nodes (69). In contrast, adjuvant does not accumulate in peripheral joints, as demonstrated in the SIA model (Lorentzen and co-workers, unpublished). It has not been determined how the oils are transported *in vivo*, but they could be transported as micelles or associated with certain lipid-transporting molecules. Alternatively, they could penetrate into cells or cell membranes where they could change membrane fluidity and modulate transcriptional regulation (70, 71). The latter possibility is supported by the fact that a common denominator of the arthritogenic alkanes, squalene and pristane is that they are soluble in cell membranes, or have a size that corresponds to fatty acids, which are the main constituents of cell membranes, whereas shorter alkanes and unsaturated alkanes lack arthritogenicity (Table 2).

The dissemination of oil or pristane leads to early local responses in lymph nodes, but also to a systemic reaction. Thus, increased blood levels of acute phase reactants, fibrinogen and α_1 -acid glycoprotein (AGP), as well as interleukin (IL)-6, can be observed already a few days after adjuvant injection (6, 72, 73). The response could be caused by a direct effect of the adjuvant in the liver but could also be indirectly triggered by cytokines such as IL-6 and IL-1/TNF α (tumor necrosis factor α). In the lymph nodes, hyperplasia develops after a few days with an enhanced expression of mRNA for pro-inflammatory IL-1 β (73), and possibly TNF α (74). *In vivo* labeling of replicating DNA with BrdU has also provided evidence that T lymphocytes proliferate at an early stage after adjuvant injection (73). This shows that some T cells respond to adjuvant injection, but it remains to be determined whether these particular lymphocytes are involved in the pathogenesis or whether there is a second or third cell population that are subsequently activated. In fact, the long time that elapses between the spread of the adjuvant (<15 min after injection) and the final outbreak of arthritis (>9 days) argues for several discrete steps in the initial pathogenesis.

Role of lymphocyte in the induction of disease onset and relapses

There is substantial evidence for a role of T cells expressing $\alpha\beta$ TCR in the induction and development of various types of adjuvant arthritis, whereas there is as yet no or very limited evidence for an arthritogenic role of B cells. Attempts to transfer arthritis from rats with adjuvant arthritis with serum or immunoglobulin (Ig)G have so far failed, in contrast to CIA, where anti-CII antibodies induce arthritis (44, 51, 75). Possibly, transfer of the IgG fraction from adjuvant-injected rats

has a suppressive effect on disease development (76). On the other hand, lymphocytes from adjuvant primed rats, which have been activated with T-cell mitogens *in vitro*, readily transfer severe arthritis to naive irradiated recipients (15, 16, 77). The arthritogenic cells are most likely CD4⁺ $\alpha\beta$ TCR⁺ T cells based on both transfer and antibody neutralization experiments (15, 31, 77). Importantly, the T cells play a crucial role at different stages of the disease as suggested by experiments showing that administration of an antibody against the $\alpha\beta$ TCR can prevent induction, delay onset and cure disease at later stages (6, 31, 68). In addition, nude rats lacking thymus and T cells are resistant (78). There is, however, no evidence so far for any particular antigen specificity or clonal expansion of the T cells that transfer disease or the T cells in the arthritogenic joints (6, 15, 77, 78). The role of CD8⁺ T cells is unclear. They are not necessary for transfer of the disease but may play a regulatory role. Thus, depletion of CD8⁺ T cells enhances development of arthritis (79), which suggests a suppressive role for CD8⁺ T cells or natural killer (NK) cells. However, the observed disease aggravation could also be dependent on an expansion of CD4⁺ T cells due to the increased space that emerges after depletion. The role of these arthritogenic lymphocytes is unclear. Autoreactivity has been considered, but attempts to reveal cellular or humoral reactivity towards heat shock proteins, CII or COMP have so far been unsuccessful. However, it is possible that the mechanism of lymphocyte activation is different in the different phases of the disease. During the initiation phase a polyclonal T-cell activation clearly occurs in the lymph nodes, whereas during the later phases there might be a more antigen-directed cause of the relapses of the disease, due to antigen exposure in inflamed joints and clonal expansion of T cells. It is important to emphasize that cartilage is a tissue exposed to the immune system and the postulated autoreactive T cells are therefore already anergized or deleted for high affinity clones. Therefore these T cells are not easily detectable by classical immune response recall assays, as has been shown for autoreactive CII-specific T cells in CIA (53). Thus, if antigen-specific T cells appear preceding and during the relapses of the adjuvant arthritis disease, it will be almost as difficult to identify them as it is in RA.

An hypothesis for disease development in adjuvant arthritis

Identification of the cause and pathogenesis of adjuvant arthritis is indeed a challenging task and classical immunological approaches, based on immune antigen-specific re-

sponses, are obviously not applicable to these investigations. It is, however, possible to analyze the problem from a disease-oriented perspective. An obvious approach is to study the critical events in the pathogenesis by comparing strains that display different susceptibility to disease and that develop different disease courses. In addition, environmental factors that affect the disease in these genetically defined strains can be studied. However, it might be worthwhile to speculate and suggest some hypotheses for the possible mechanisms based on the characteristics defined so far, which may give leads to further investigations. The crucial questions to be answered are:

- Why do endogenous and normally harmless adjuvants like squalene and pristane induce inflammation?
- Why are peripheral joints attacked and not other tissues?
- Why is the disease relapsing as in PIA and AvIA?

We believe that the answers to all of these questions must involve the $\alpha\beta$ TCR-expressing T cell. This does not necessarily involve clonal expansion of highly proliferative and auto-antigen specific T cells. Rather, during the induction phase the introduced adjuvant will skew the T-cell populations by polyclonally expanding self-reactive T cells. These are already primed T cells, which may be low affinity T cells and T cells ignoring self antigens, which have been expanded and maintained through low affinity antigen interactions. This sudden expansion of self-reactive T cells cannot be completely counteracted by already existing regulatory T cells. The existence of such cells has been convincingly demonstrated in both mice and rats (80–84). Depletion of them will lead to the development of inflammation in certain organs such as the stomach, liver, pancreas and thyroid, but not in the joints. Still, the induction of disease is tissue-specific and no auto-antigen/s have so far been identified. It is thus likely that the adjuvants skew the T-cell population in a different way to explain the joint specificity, determined by the structure or function of peripheral and cartilaginous joints. So what is special about joints?

Joints are easily accessible for the immune system due to the vascularization and lymph drainage of the joint capsule – the synovial tissue. In fact this tissue cleans the blood of circulating debris of various sorts and synovial macrophages are easily activated in infectious and inflammation disorders. The joint cartilage, on the other hand, is not vascularized and it is only indirectly exposed to the immune system, leading to a partial but not complete tolerance against the cartilage proteins. A third unique feature of joints is that they are repeat-

edly traumatized by locomotion and scavenging. A fourth feature of the joints is that they are repaired after having been damaged or destabilized. The repair leads to formation of new cartilage and bone and sometimes to deformations of the joints – a physiologic process seen in the fingers of young rock climbers and in almost all elderly people. Thus, the joints have the capacity to both destroy and rebuild themselves, the synovial tissue cells are repeatedly stressed and antigens are released in a physiologic process. These features, taken together, suggest that the T cells already have been exposed to joint antigens and been stimulated to an extent that provides survival but not full aggressiveness (due to clonal anergy or deletion of high-affinity clones). Such T cells may also play a role in the physiologic synovitis that often occurs in response to infections. The immune system is held in balance by various mechanisms, probably involving both tolerance and regulation. The sudden appearance of large amounts of antigens in an improper context, as will be the effect of a subcutaneous injection, will destroy this well-developed physiologic balance. The activation of T cells is apparently detrimental and the questions that can be investigated are the nature and specificity of these T cells.

The same T cells, however, may not be responsible for relapses of the disease as seen in some models like the PIA model in DA rats. In fact, the chronic process seems to be determined by a unique set of genes, which suggests that another hypothesis than the one postulated for the initiation of the disease needs to be considered. One possibility is that once the inflammatory attack on the joints is prolonged it will elicit new attempts by the body to counteract destruction. Normally, the body would respond by trying to establish a ceasefire with the intruding inflammation causing pathogen. In this case the ceasefire will be with its own autoreactive T cells in the absence of a pathogen. This will lead to a long-term and repetitive stimulation of autoreactive T cells, in response to joint antigens and neoepitopes exposed in an inflammatory context. Thus, we suggest that the autoreactive lymphocyte repertoire will be gradually more limited and oligoclonally expanded and that reactivities to joint antigens by partially tolerized lymphocytes may appear. Such a process is compatible with observations in RA (85–89). These T cells may not necessarily be pathogenic but they could be so in genetically predisposed individuals or after certain environmental challenges.

Genetic control of adjuvant arthritis models

The adjuvant arthritis models are in general very reproducible and in most cases highly penetrant in the DA strain. The DA

rat is highly susceptible not only to the pure adjuvant type of arthritis models (6, 31, 68, 78) but also to models involving bacterial cell wall fragments such as MIA (90), to cartilage protein-induced arthritis (i.e. CIA) (36, 37, 57) as well as to other autoimmune diseases such as relapsing experimental allergic encephalomyelitis (EAE) (91). Many other rat strains have been tested for disease susceptibility and were found to vary dramatically. Most strains are resistant to OIA and only the DA and the DXEA strains develop a mild acute arthritis (33, 34, 40). The DXEA strain contains DA genes since it is a recombinant inbred of E3 and DA. In contrast, only a few strains (E3 and DXEC) are resistant to PIA and AvIA and the DA strain develops a chronic relapsing disease course (6, 78). In general, F1 hybrids with DA are susceptible to PIA (but not OIA) but the disease is generally milder and displays a lower penetrance, indicating that the environmental factors need to be carefully controlled as is the case for most complex disease models. The most important environmental factors seem to be stress effects, infections, nutrition, light cycles and hormone cycling. Also, factors like gender will influence the linkage to certain loci dramatically. Nevertheless, several gene segregation experiments have been performed and many loci associated with disease have now been identified and confirmed by breeding of congenic strains.

Role of the major histocompatibility complex

The role of the MHC region in many of these models (OIA, AvIA, PIA and SIA) has been addressed using already established congenic strains on the LEW or DA background as well as in F2 crosses (6, 33, 40, 78, 92). Thus, studies of PIA have confirmed an MHC association (denoted Pia1) in both congenic strains and F2 crosses (6, 92). The most susceptible haplotype in MHC congenic LEW strains was found to be the RT1-f (6), which is also associated with the genetic control of AvIA (78) and type XI CIA (39). Interestingly, the association was observed only for the chronicity of the disease, whereas no significant association was seen with other parameters, like the day of arthritis onset or incidence. It is a surprising finding that PIA, like all other adjuvant arthritis models, is associated with the MHC. In cartilage protein-induced models, such an association is expected since the MHC class II genes are likely to control the immune response elicited by the inducing antigen. In fact, work performed in the mouse has made it possible to identify the gene in the MHC region that is associated with CIA in mouse. This is the *q* allele of a DQ class II gene (the classical nomenclature is A in the mouse and B in the rat) and its role in CIA is most likely related to its capacity to bind the immunodominating

CII peptide (positions 260–270) (93). Interestingly, the peptide-binding pockets of human class II molecules that are associated with RA, the “shared epitope”, present in the DR4 (DRB1*0401/DRA) molecule, resembles the mouse DQ *q* allele. Mice expressing human class II molecules of the shared epitope type (DR4 and DR1) are susceptible to CIA and bind a very similar peptide 261–273 ((94–96) and reviewed in (97)). In the rat models the relevant peptides from autologous type II collagen have not yet been identified but it seems likely that these will be found and that a corresponding structural relationship, as in the mouse, will be revealed. The rat class II molecules have been sequenced and from the results it can be inferred that the genetic association to both CIA and C^MIA is caused by a DQ molecule as in the mouse (37, 98). However, with adjuvant arthritis models these assumptions cannot be made as easily, since there is no exogenous immunogen, and there are not as strong arguments for the involvement of class II genes in AA. Although it is likely that CD4⁺ αβTCR⁺ T cells play a crucial role in adjuvant arthritis, it has not yet been possible to isolate antigen-specific T cells or to provide evidence for MHC class II restriction.

It is important to emphasize, however, the MHC congenic strains, used in experiments to find associations with MHC, are not well controlled since they have been used for breeding in different laboratories for decades and was not originally made with marker-assisted technology and it is likely that they contain non-MHC contaminations. The use of separate MHC haplotypes and the same MHC congenic region on different gene backgrounds such as both LEW and DA does, however, increase the likelihood of a role for MHC. However, although the linkage in adjuvant arthritis models has also been seen in MHC F2 crosses, it is clearly weaker than has been observed in the CIA or MIA models (37, 59, 90, 92). The difficulties in demonstrating strong MHC associations in the adjuvant arthritis models could possibly be explained by suppressive interactions with other segregating genes or by the use of haplotypes with only small differences in phenotypic effects. Another explanation could be that the MHC genes in the adjuvant arthritis models control a later phase of the disease as compared with CIA. Thus, the MHC linkage remains to be confirmed using better-characterized MHC congenics. Moreover, even if MHC linkage is confirmed, it remains to be conclusively shown that the MHC class II genes are involved. Although indirect evidence argues for a role of MHC class II genes, it is likely that there are a number of other genes within the MHC that will affect adjuvant arthritis. This remains, however, to be demonstrated. One of the more important challenges is therefore to delimit the MHC region by

congenic breeding and to clone the alleles associated with adjuvant arthritis to establish their identity as well as their role in the disease.

Role of the acute phase response

Studies of the acute phase response in the adjuvant arthritis models are relevant for many reasons. This response is a response of the innate immune system, which is effectively triggered by the adjuvant injection. The response is normally acute, but during a chronic inflammatory response many of the "acute" phase proteins also become persistently up- or downregulated. It is therefore likely that they are involved in the pathogenesis. In addition, the hepatocytes in the liver are the main producers of the acute phase proteins. Thus, the acute phase proteins are measurable in circulating blood and are therefore easy to monitor in the experimental animals. Three key regulators of the acute phase response are IL-6, IL-1 and TNF α . The acute phase proteins can be divided into type 1 acute phase proteins, regulated by IL-1 and TNF α (α_1 -acid glycoprotein and serum amyloid A), and type 2 acute phase proteins, regulated by IL-6 (fibrinogen, α_1 -antitrypsin and α_2 -macroglobulin) (99). In rat models for arthritis three acute phase proteins and one cytokine (fibrinogen, α_1 -acid glycoprotein, α_1 -inhibitor₃ and the cytokine IL-6) have been studied by linkage analysis (72).

Many QTLs (quantitative trait loci) were found, some of which coincided with previously identified loci for clinical arthritis (PIA). Whether this means that the acute phase is secondary to the arthritis inflammation and just reflects a disease symptom or is separately regulated by the same genes is difficult to conclude from these results and further studies using congenic strains are required to provide an answer. The results clearly show that inflammatory response proteins are useful for the diagnosis and phenotyping of the animals. Identification of subphenotypes like the acute phase proteins will facilitate further breeding of congenic strains and will hopefully in the end allow the cloning of the genes that are involved in the pathogenesis of adjuvant arthritis.

Genetic dissection of OIA

As described above, different rat strains differ widely in their susceptibility to OIA. It should thus be possible to identify genetic loci associated with disease susceptibility by analyzing progeny from the F2 generation or from backcrosses between susceptible and resistant rat strains. Based on experiments with MHC congenic DA rat strains, it was reported that the MHC haplotypes RT1n (33) and RT1h (40) abrogated or ameliorated the development of OIA. However, it was also demonstrated

that non-MHC genes in the DA rat determine arthritis development, since LEW.1AV1 and PVG.1AV1 rats are resistant although they share the congenic fragment with the RT1av1 haplotype (40, 100). To map the chromosomal location of susceptibility genes, a genome-wide linkage analysis was performed, employing progeny from a (DAXLEW.1AV1)F2 intercross (101). LEW.1AV1 was selected as the resistant parental because it is MHC identical to DA. In this way, the MHC (designated Oia1) would not contribute to clinical variance in the F2 offspring, thus optimizing the detection of non-MHC QTLs. The linkage analysis identified two major QTLs determining arthritis susceptibility, one on chromosome 4 (Oia2) and one on chromosome 10 (Oia3). Both regulated disease penetrance and severity in a DA-additive and DA-recessive fashion, respectively, and both loci were reproduced in a backcross between DA and LEW.1AV1. Together, the two QTLs accounted for a large portion of the clinical variance in the intercross. Thus, offspring that were homozygous for LEW.1AV1 alleles at both Oia2 (D4Mgh10) and Oia3 (D10Mgh1) were resistant, whereas those homozygous for DA alleles at both loci were susceptible. Interestingly, many of the Oia2/Oia3 DA homozygous offspring developed more severe arthritis than DA, indicating a genetic contribution from the resistant LEW.1AV1. A similar modulation of the phenotype by alleles from the resistant strain was observed when an intercross population was examined in another adjuvant arthritis model, induced with squalene (68). Thus, 21% of the affected (DAXLEW.1AV1)F2 rats developed chronic relapsing SIA, although DA rats only develop a monophasic disease, and LEW.1AV1 is almost resistant. In the SIA experiment, the limited number of progeny did not allow for a linkage study of chronicity. However, both Oia2 and Oia3 could be reproduced as linked to susceptibility and linkage was also found to increased plasma fibrinogen levels before arthritis onset.

The next step was to determine whether the two QTLs confer to susceptibility after being transferred to other strains by selective breeding. DA alleles from the Oia3 locus were transferred to LEW.1AV1 rats (102) (Fig. 1). The penetrance of OIA in Oia3 congenic rats was only 13% (2/15). However, a high penetrance was observed after induction of arthritis with squalene, which is a more potent adjuvant oil. A penetrance of 79% was observed in the congenic strain compared to 13% in LEW.1AV1 (22/28 vs 3/23, (102)). These results demonstrate that susceptibility and resistance depend on the disease trigger. This property can be used as a tool for further dissection of experimental arthritis. More importantly, the results demonstrate for the first time that a single QTL, other than the MHC, can transfer arthritis susceptibility. The clinical

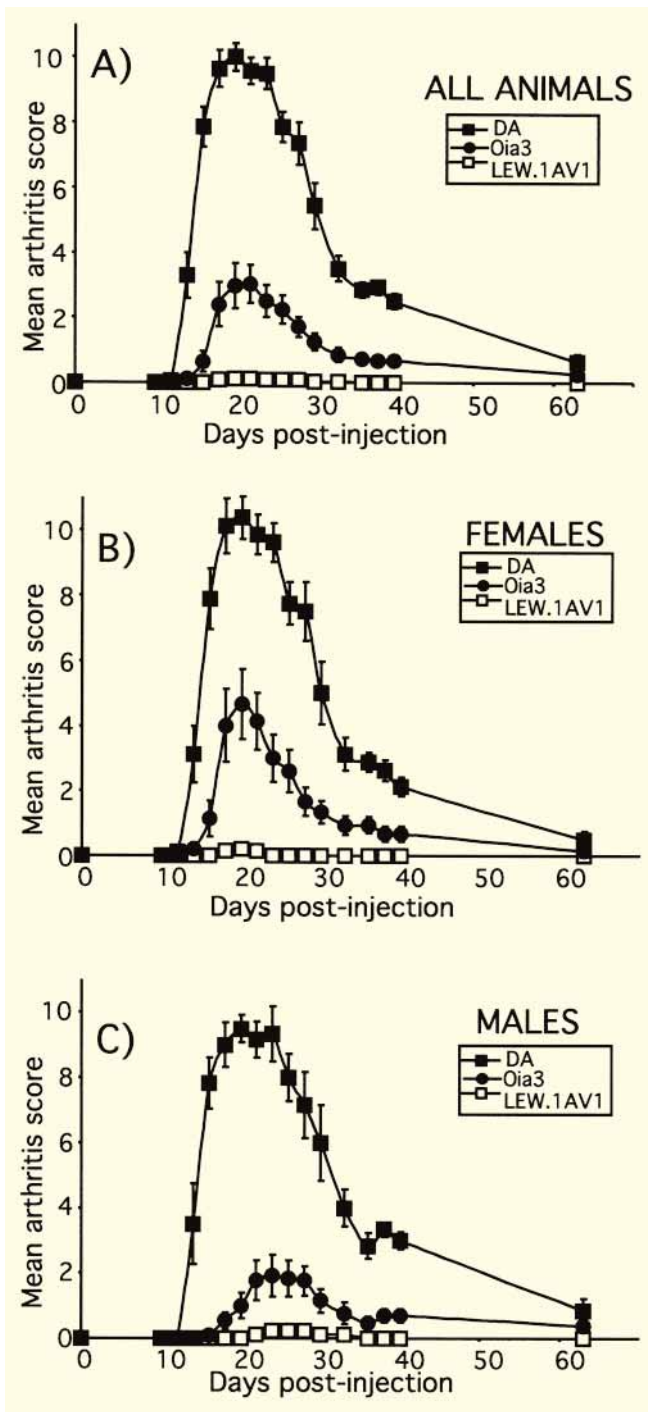


Fig. 1. Development of SIA, illustrated as mean scores for DA, LEW.1AV1 and *Oia3*-congenic rats vs. days post-injection with squalene. ■ DA animals. ● *Oia3*-congenic animals. □ LEW.1AV1 animals. Standard errors of the mean are indicated as bars. A more severe arthritis is observed in the *Oia3*-congenic strain compared to the parental LEW.1AV1 strain. Adapted from (68, 102).

symptoms of SIA in *Oia3* congenic rats occurred together with other clinical phenotypes, such as infiltration of T cells into joints, increased blood levels of acute phase proteins, and reduced weight gain. To map the arthritis regulating *Oia3* gene(s), several subcongenic strains were produced and tested, using the SIA model. A gender influence was observed, as a smaller congenic fragment mediated susceptibility in females only, whereas a larger fragment also conferred disease association in males. These results show that at least two genes are involved in the susceptible phenotype. Interestingly, a region containing the same genes was reported to be associated with CIA (*Cia5*) (57, 62) using DA as the susceptible parental, suggesting that they also are involved in this disease model. For the *Oia2* locus, congenic strains have been made in all combinations between DA, LEW.1AV1 and PVG.1AV1. Preliminary data indicate a 15% penetrance of OIA in LEW.1AV1 rats congenic for the DA *Oia2* haplotype (2/13), and a 46% penetrance in the SIA model (6/13), similar to the results obtained with the *Oia3* congenic rats. There are also preliminary data (L. Backdahl, U. Ribbhammar, J. C. Lorentzen), suggesting that congenic DA rats with a 70 cM chromosome 4 fragment from PVG.1AV1, which contains the *Oia2* locus, are resistant or less susceptible to OIA, SIA, PIA, MIA and CIA. In CIA the isotype profiles of auto-antibodies appeared to be changed, suggesting that the locus has an influence on the qualitative regulation of autoimmunity. A similar phenomenon has been observed in EAE, in which the *Oia2* region most likely explains the reported linkages to auto-antibody isotype profiles in a cross between DA and PVG.1AV1 (103). *Oia2* seems to be involved in many different arthritis models. Thus, linkage has been reported to the similar chromosomal region in PIA (*Pia7*) (92), and CIA (*Cia13*) (60), in both cases using DA as the susceptible strain, and with a similar inheritance pattern and subphenotype linkage. The susceptibility allele in the *Oia2* locus of DA is not present in E3 and BN, because these were the resistant strains used in PIA and CIA, respectively. It also seems to be absent from LEW and PVG because *Oia2* from DA is linked to OIA when LEW.1AV1 and PVG.1AV1 were used as resistant strains (65, 104). However, the allele may be shared by F344, since linkage to *Oia2* was not identified in a cross between DA and F344, used for analysis of MIA and CIA. Interestingly, a locus in a syntenic position has been identified in analysis of CIA in the mouse (*Cia6* on MMU6) (105), indicating that the region contains polymorphism of importance for arthritis also in other species.

That *Oia2* and *Oia3*, in combination, have an additive effect in F2 offspring (101), is supported by preliminary data in a

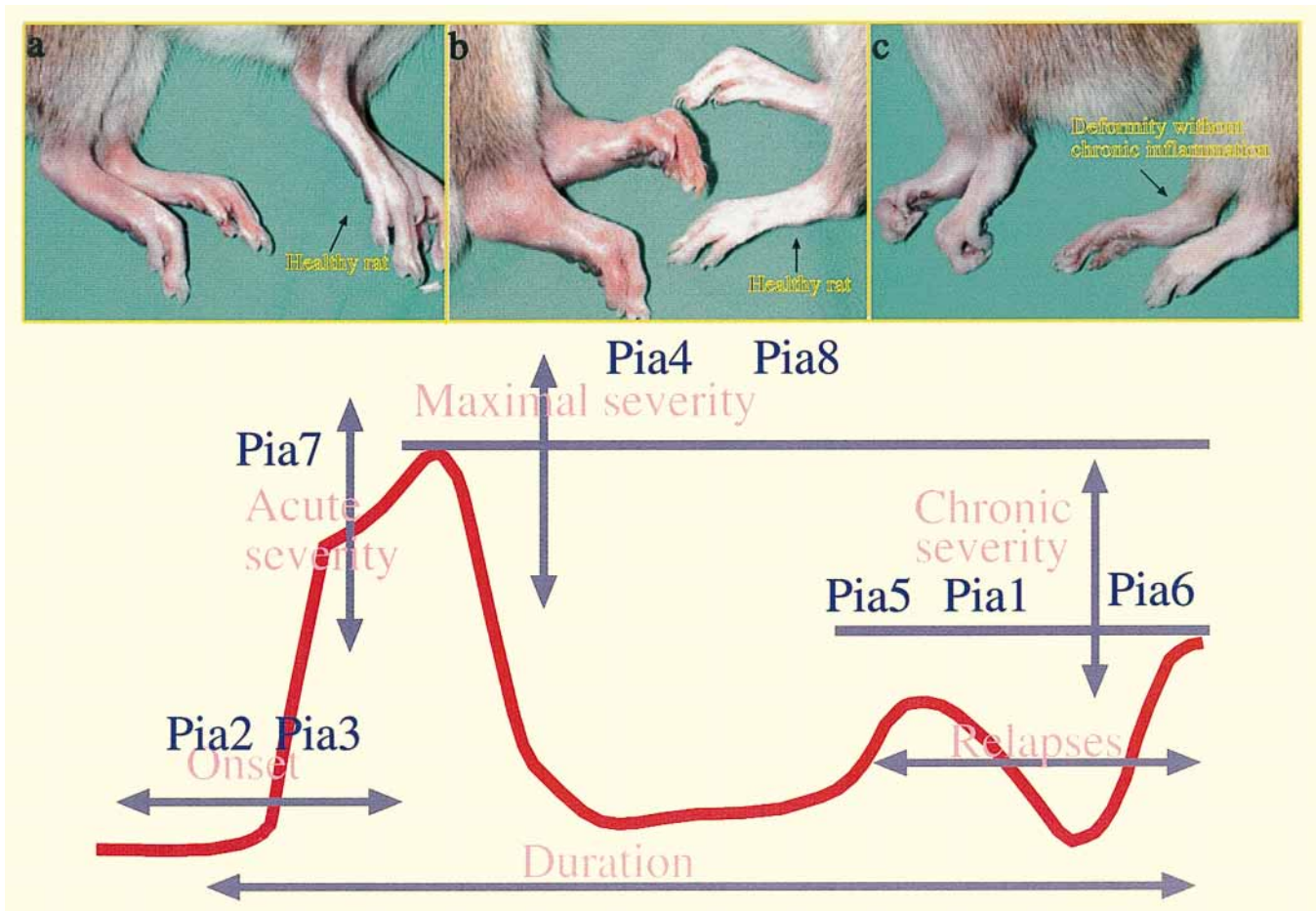


Fig. 2. Typical disease course of PIA. The different subphenotypes are indicated together with loci associated with them. a) Hind paws with acute arthritis and normal hind paws. b) Hind paws with severe arthritis

and normal hind paws. c) Hind paws with chronic active arthritis and hind paws with healed arthritis (chronic inactive arthritis).

double congenic strain. Thus, LEW.1AV1 carrying both *Oia2* and *Oia3* from DA became 100% susceptible to OIA (8/8), compared to 2% in LEW.1AV1 (1/63, as summarized from several reports (40, 100, 101, 106)). This indicates an interaction between genes in the two chromosomal regions, since the penetrance was only 13–15% in congenic strains that contained only one of the two susceptibility loci (2/15 and 2/13, respectively). In RA, linkage has been reported to human chromosomal regions being syntenic to *Oia2* (12p) (107, 108) and *Oia3* (17q) (108, 109). We thus conclude that the *Oia2* and *Oia3* regions are likely to contain genes involved in the pathways that lead to arthritis susceptibility both in animals and in humans.

Genetic analysis of different phases of the PIA model
The PIA model is valuable since it is a model that closely mimics RA (Table 1). A main advantage is its chronic relapsing

course, allowing genetic linkage analysis to be performed during different phases of the disease. Both PIA and AvIA develop as chronic arthritis in most strains, among which DA shows the most aggressive disease course (6). Although other adjuvant arthritis models are monophasic even in the DA strain, as discussed above, a chronic arthritis may develop in F2 rats emphasizing that the impact of the environmental insult depends on combinations of genetic factors.

Using the PIA model in the DA rat, the arthritis develops suddenly and dramatically 2 weeks after pristane injection. An episode of severe and destructive arthritis in the peripheral joints follows and gradually subsides 3 weeks later. However, starting at around 6–8 weeks after pristane injection, a chronic relapsing disease develops which can reach almost as high severity as during the first arthritic episode and does not subside. Based on the disease course, a number of subphenotypes have been defined and are illustrated in Fig. 2.

Table 3. Mapped loci associated with PIA

Locus	Chr	Arthritis phenotype	Cross/linkage	Marker	Inheritance ^a	Confirmed in congenic strains ^b	QTLs in other rat models
<i>Pia 1</i>	20	Chronicity	MHC congenic	D20mgh5	E3 dom	p<0.01	<i>Aia1, Cia1, Oia1, Eae1</i>
<i>Pia 2</i>	4	Onset	E3×DA LOD 3.9	D4mgh14	E3 dom F	p<0.006	<i>Aia2, Eae11</i>
<i>Pia 3</i>	6	Onset	E3×DA LOD 4.5	D6WOX5	E3 dom	–	<i>Eae9</i>
<i>Pia 4</i>	12	Severity	E3×DA LOD 8.4	D12WOX14	DA rec	p<0.0001	<i>Cia12, Eae5, Eau2</i>
<i>Pia 5</i>	4	Chronicity	E3×DA LOD 4.5	D4wox20	DA rec M	p<0.09	<i>Aia3, Cia3, Eau1</i>
<i>Pia 6</i>	14	Chronicity	E3×DA LOD 4.9	D14Cсна	DA rec	p<0.01	<i>Eae10</i>
<i>Pia 7</i>	4	Early severity	DXEC×DA LOD 4.9	D4rat60	DA rec	p<0.09	<i>Oia2, Cia13</i>
<i>Pia 8</i>	1	Severity	DXEC×DA LOD 4.7	D1mgh2	E3 dom F	p<0.05	

^a dom=dominant, rec=recessive, add=additive. M=linkage in males, F=linkage in females.

^b the p-value does not necessarily reflect the degree of penetrance but rather the statistical power (number of tested animals).

In order to genetically map the loci associated with these various disease subphenotypes we made crosses between the susceptible DA strain and the resistant E3 strain (110) (Table 3 and Fig. 2). In addition, a series of recombinant inbred strains with genes inherited from both DA and E3 were characterized. One of these strains, DXEC, was found to be resistant to arthritis in spite of having approximately half of the susceptibility loci from DA (9). Thus, the DXEC strain was also used in crosses with the DA (92).

1) Genetic control of arthritis onset

A characteristic of the PIA model is that the disease starts with a sudden onset with arthritis in peripheral joints. The phenotype is clear and it is easy to determine the day when the joints start to get red and swollen, which is followed by a period when the disease will develop into full-blown arthritis of varying degree. The time of onset in the parental DA rat is between 10–14 days after the injection of pristane, while the E3 rat is totally resistant. These facts made it possible to define a phenotype as the day of onset after pristane injection. In the intercross between E3 and DA, two loci are associated with the day of onset. The identified QTLs were named *Pia2* (chr 4) and *Pia3* (chr 6). A surprising finding was that the susceptibility allele in both cases was derived from the resistant E3 strain. This indicates that the E3 rat has a genetic predisposition to start an arthritis outbreak but lacks the severity genes required for development of a clinical observable disease. The DA rat apparently lacks these onset-regulating alleles and it is possible that DA animals with these onset alleles might develop arthritis even earlier or spon-

taneously. However, no spontaneous disease of high frequency has yet been observed in congenic strains although the disease develops faster in *Pia2* congenic rats. The original observation that *Pia2* was sex linked, since the linkage was seen only in females (110), was also observed in the congenic strain. Interestingly, the *Pia2* locus has also been identified in the MIA model but not in the CIA model, using the same strains in the crosses; the DA and F344 strains (59). In addition, the locus was also found in the EAE model for MS using an (E3×DA)F2 cross and the same inheritance and subphenotype patterns were observed as in PIA (the locus was denoted *Eae3*) (111). Taken together, the data show that there are genes that control onset of inflammatory disease irrespective of disease type or target tissue.

2) Genetic control of arthritis severity

The severity of arthritis in animal models as well as in patients with RA can be quantified by evaluating the numbers of affected joints and the severity of the swelling and redness of the joints. In animal models the degree of cartilage and joint destruction can be addressed through histopathologic analyses of affected joints and also through measurements of circulating COMP, most likely reflecting the degree of cartilage erosion (112). In the case of PIA the mean scoring value for each day after pristane injection, the maximal mean scoring value, and levels of circulating COMP were used as phenotypes. By using these arthritis severity measurements as quantitative traits in the linkage analysis, three loci that regulate severity were identified: *Pia4* (chr12) and *Pia7* (chr4) were inherited as DA-recessive loci associated with arthritis sever-

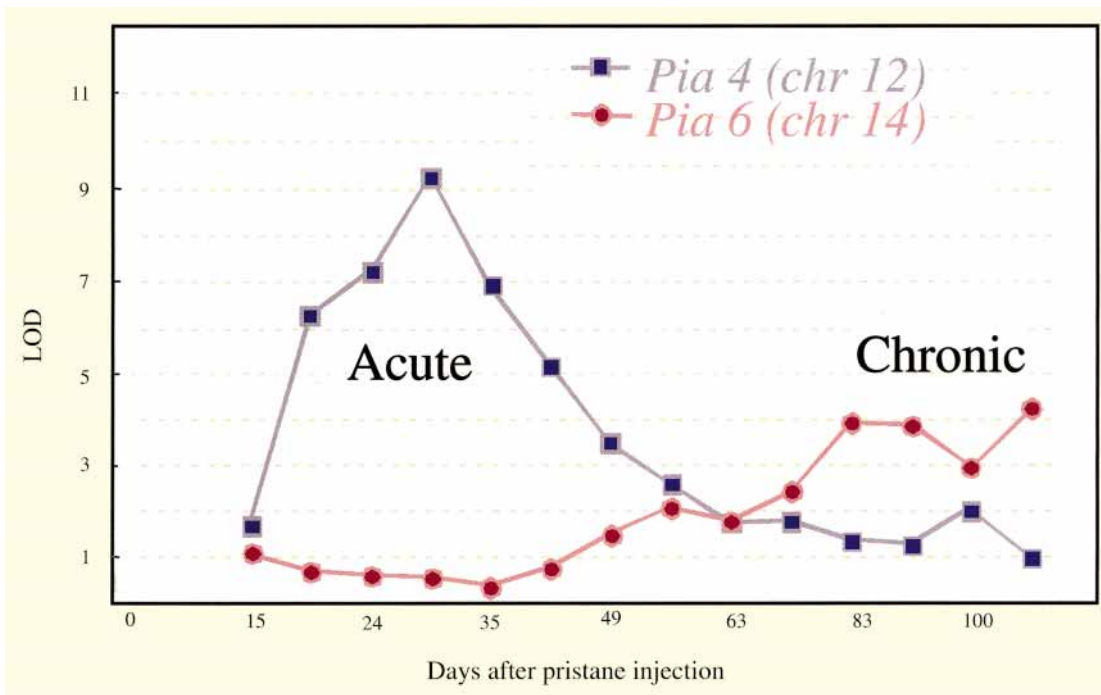


Fig. 3. LOD scores for *Pia4* and *Pia6* calculated from the mapping data of an (E3×DA)F2 cross, published in (110).

ity, or inherited as E3-derived dominant protective loci, whereas *Pia8* contained an E3-derived susceptibility locus. Interestingly, these different loci were not associated with the same phase of the disease or the same specific severity subphenotype. The *Pia7* locus is associated with a very early phase, with acute severity of arthritis developing only within a few days after onset, whereas the *Pia8* locus reflects severity several weeks later. The *Pia4* locus shows the strongest association at the peak of the severity or, rather, seems to be associated with joint destruction (Fig. 3). This is apparent using COMP as a phenotype. An effect primarily on the severity has been confirmed in *Pia4* congenic strains as illustrated in Fig. 4. In the same region as *Pia4* a susceptibility locus for EAE (*Eae5*) has also been identified in a cross between E3 and DA (111). Interestingly, the *Eae5* locus also regulates disease severity in a DA-recessive pattern but it seems to have stronger association with the relapsing phase in the EAE model than was observed for *Pia4* in the PIA model. It seems that both *Pia4* and *Eae5* are associated with target-tissue destruction rather than with destruction at a particular time phase of the disease. *Eae5* gave a LOD (logarithm of odds ratio) score as high as 13 with the severity phenotype to be compared with a LOD of 8 for *Pia4*, which suggests that the locus contains gene(s) common to both diseases. Loci linked to experimental autoimmune uveoretinitis (*Eau2*) and CIA (*Cia12*) have also

been detected in the same region using different rat strains. If these linkages involve the same polymorphism it indicates that alleles at this locus are involved in many different inflammatory diseases. This could also include the mouse, in which a syntenic region on chromosome 5 has been found to be associated with arthritis severity of Lyme disease (113).

3) Genetic control of arthritis chronicity

It was found that loci on chromosome 4 (*Pia5*) and 14 (*Pia6*) were associated with active arthritis during the chronic stage. In the DXECxDA cross we also found linkage to *Pia1* (MHC) in the beginning of the chronic phase, confirming the earlier observation in LEW MHC congenic strains (see above). Interestingly, these loci were not associated with arthritis onset or severity at earlier stages of disease. However, each QTL seems to control specific variants of the chronicity. The *Pia5* locus is associated with histopathologic inflammation score of joints at very late stages in males but not in females. In preliminary experiments we observed that *Pia5* in congenic strains also has effects that occur exclusively in males (B. C. Holm, L. Svelander, A. Bucht, J. C. Lorentzen, unpublished observation). Interestingly, *Pia5* is located in the same region as a previously identified locus controlling CIA (denoted *Cia5*) (57) but the different time courses of the phenotype indicate that different genes may be involved. The *Pia6* locus is linked

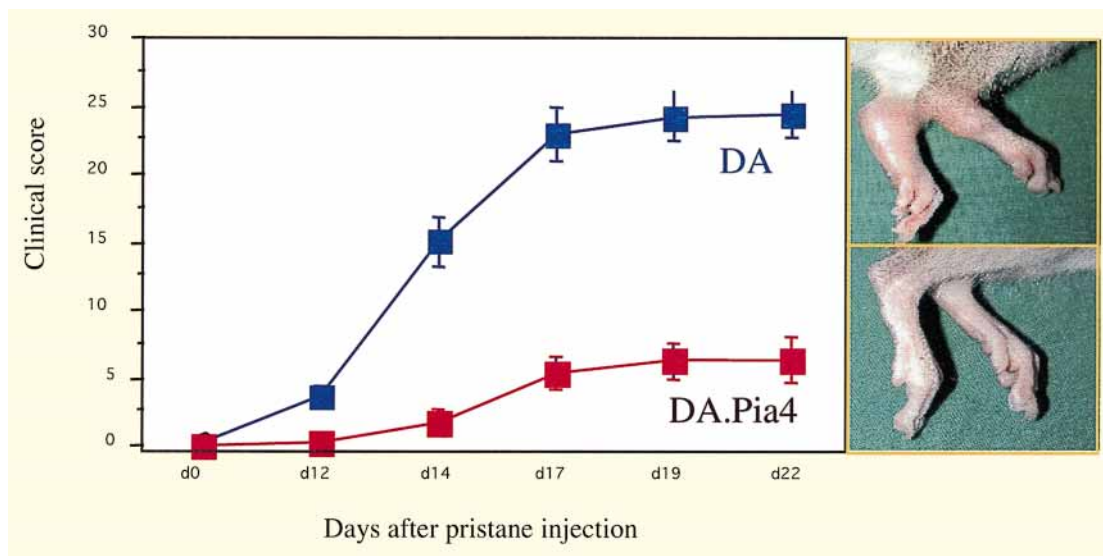


Fig. 4. The disease severity is controlled by *Pia4*. In *Pia4* congenic rats, the E3 fragment of *Pia4* has been introgressed on the DA background and

the *Pia4* heterozygous rats are compared with the wild type DA rats for development of PIA.

to clinical scores and is significant from the start of the chronic relapsing disease period, i.e. from approximately 6 weeks after pristane injection. The *Pia 6* locus has also been observed in EAE and controls a similar subphenotype (relapsing disease) as in arthritis (111).

Clearly, the identified gene regions associated with chronic disease contain genes that have a unique importance in the chronic development of disease. As such, they will hopefully provide unique information on the pathology of chronic diseases.

Concluding remarks

The induction of arthritis in peripheral joints using adjuvant seems to be unique for the rat. The reason for this species-specificity is unclear but the model is helpful for studies of the complex pathways leading to arthritis. The disease is induced by immunostimulation in the absence of exogenous

antigens, but it is still dependent on the activation of $\alpha\beta$ T cells and is controlled by the MHC region. Therefore, immune recognition of endogenous structures is likely to be involved and could promote the development of a self-perpetuating chronic relapsing disease. Genetic analysis shows that the different phases of the disease are controlled by different genes and thereby involve different pathogenic pathways. Further analysis of these models, together with models induced with various cartilage proteins like type II collagen, are therefore likely to provide essential information on how chronic inflammatory diseases develop. This is of great importance since, as basic scientists, we often forget that the human autoimmune diseases, like RA, are chronic in nature. As clinicians we tend to overemphasize the possibility of finding the clues to the disease mechanisms from signs and symptoms in individual patients or even from genetic and epidemiologic analysis of patient cohorts. The animal models provide us with exceptional tools for precise genetic analysis of the pathways that lead to complex diseases like RA.

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