Review

The determinants of susceptibility/resistance to adjuvant arthritis in rats

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Abstract

Adjuvant arthritis (AA) serves as an excellent model for human rheumatoid arthritis. AA is readily inducible in certain rat strains, but not in others. Susceptibility/resistance to AA is determined by multiple factors. Among the genetic factors, both MHC and non-MHC genes contribute to arthritis susceptibility, and specific quantitative trait loci show association with the severity of the disease. Differential T-cell proliferative and cytokine responses, as well as antibody responses, to heat-shock proteins are evident when comparing AA-susceptible and AA-resistant rats. In addition, neuroendocrine factors and the housing environment can further modulate arthritis susceptibility/severity in particular rat strains.

Introduction

Adjuvant arthritis (AA) is inducible in susceptible rat strains, such as the Lewis (LEW) rat, by a single subcutaneous injection of heat-killed *Mycobacterium tuberculosis* H37Ra (Mtb) in oil. AA has been used extensively as an animal model of human rheumatoid arthritis (RA) for studies on the disease pathogenesis and for testing of new products for their therapeutic efficacy [1]. Various outbred and inbred rat strains differ in their relative susceptibility to AA (Table 1). Similarly, the prevalence of RA differs significantly among human populations living in different geographical regions of the world, and even among subpopulations within the same region [2]. Conducting well-controlled studies to unravel the mechanisms underlying the disease susceptibility in RA, however, is difficult for multiple reasons – including the genetic heterogeneity of human populations and the differences in the environmental influences. In this regard, studies in animal models of arthritis serve an invaluable purpose by providing information that is directly relevant to human RA.

In the past 10 to 15 years, significant advances have been made in unraveling the mechanisms involved in the initiation of AA as well as the regulation of AA. Studies comparing the physiological characteristics as well as the immune responsiveness of AA-susceptible rat strains versus AA-resistant rat strains have provided critical insights into the disease process and have thereby contributed to these advancements. In the present review we highlight the major factors determining the susceptibility/resistance to AA (Table 2). In a few places, studies from other models of arthritis are also included.

The differential susceptibility to AA of inbred rat strains bearing different MHC haplotypes (for example, LEW rats and Brown Norway (BN) rats) as well as those possessing the same MHC haplotype but having disparate non-MHC (background) genes (for example, LEW rats and Wistar–Kyoto (WKY) rats) (Table 1) underscores the significance of genetic factors in determining susceptibility/resistance to AA. These genetic factors mediate their effect in part via influencing the quantitative and qualitative aspects of immune response to the disease-related antigens (Table 2). Superimposed on this genetic predisposition is the modulation of disease susceptibility by hypothalamic–pituitary–adrenal (HPA) axis activity and microbial agents.

Genetic linkage studies for adjuvant arthritis-linked quantitative trait loci

Studies in experimental models have identified many quantitative trait loci (QTLs) that show a significant association with arthritis susceptibility and severity [3-6]. At least 52 QTLs have been identified in different rat models of arthritis, and these QTLs cover approximately 54% of the total rat genome

AA = adjuvant arthritis; AO = Albino Oxford; Bhsp65 = mycobacterial heat-shock protein 65; BN = Brown Norway; DA = Dark Agouti; F344 = Fischer F344; GN = autoimmune glomerulonephritis; HPA = hypothalamic–pituitary–adrenal; hsp = heat-shock protein; IFN = interferon; IL = interleukin; LEW = Lewis; LNC = lymph node cells; MHC = major histocompatibility complex; mRNA = messenger RNA; Mtb = *Mycobacterium tuberculosis* H37Ra; QTL = quantitative trait locus; RA = rheumatoid arthritis; Rhsp65 = rat heat-shock protein 65; ROS = reactive oxygen species; Th1 = T-helper type 1; TLR = Toll-like receptor; TNF = tumor necrosis factor; WKY = Wistar–Kyoto.

Table 1

Adjuvant arthritis-susceptible/resistant rat strains

Strain	Susceptibility to AA	MHC haplotype	References
Inbred rats			
Lewis[a]	+	RT-1^{l}	[21-24]
Dark Agouti	+	RT-1^{av1}	[45]
Piebald Viral Glaxo	+	RT-1^{c}/RT-1^{av1}	[65]
Brown Norway[b]	+/–	RT-1^{n}	[23,33]
Fischer F344[c]	+/–	RT-1^{1vl}	[59,60]
Wistar–Kyoto	–	RT-1^{l}	[24]
Wistar Albino Glaxo	–	RT-1^{u}	[42]
Buffalo	–	RT-1^{b}	[41]
Albino Oxford	–	RT-1^{u}	[45]
Outbred rats			
Sprague Dawley[d]	+	–	[66]
Holtzman[e]	+	–	[41]
Wistar[f]	+/–	–	[31,55,56]

MHC, major histocompatibility complex. [a]Generally, male and female rats are comparable in their incidence of and the course of adjuvant arthritis (AA). In one study, however, higher sensitivity of female rats over male rats for complete Freund's adjuvant-induced inflammation and hyperalgesia has been reported [67]. [b]Reported to be AA resistant in one study [23], but susceptible (males susceptible with moderate level of severity, but females resistant) in another study [33]. [c]Fischer F344 rats can develop AA when bred and kept in a germ-free or barrier facility, but acquire resistance when bred and kept in a conventional environment. [d]Outbred rats; males develop AA of much greater severity than female rats. [e]Originally derived from the Sprague Dawley rat. [f]Outbred rats with lines of rats showing susceptibility or resistance to AA.

[6]. These QTLs are distributed among various chromosomes (including the X chromosome), except for chromosomes 11, 13 and 17, but at present there is no information about QTLs on the Y chromosome [6]. Some QTLs on autosomes show gender preference.

QTLs for one animal model of arthritis (for example, AA) overlap with and/or show homology with QTLs for other experimental models of arthritis (for example, collagen-induced arthritis) as well as for human RA. In addition, QTLs for arthritis susceptibility overlap with those identified for several other autoimmune diseases, including experimental autoimmune encephalomyelitis, insulin-dependent diabetes mellitus, autoimmune uveitis, lupus, and inflammatory bowel disease [4,7]. Specific arthritis-related QTLs show a strong correlation with different phases of the disease (onset, peak, and recovery) as well as with pathological features of arthritis (pannus formation, cartilage damage or antigen-specific antibody levels). The QTLs also include the genes for cytokines and cytokine receptors, antigen processing and presentation, and hormonal balance.

For AA, *Aia1* on chromosome 20 and *Aia2* and *Aia3* on chromosome 4 show a strong association with arthritis severity [4,5]. *Aia1* includes the rat MHC (RT.1), and two of the above three loci are common with collagen-induced arthritis in rats – namely *Aia1*/*Cia1* and *Aia3*/*Cia3* [4,5]. The QTLs *Aia1*, *Aia2* and *Aia3* from AA-resistant Fischer F344 (F344) rats reduce arthritis severity as expected, but surprisingly *Aia4* from F344 rats enhances arthritis severity when compared with AA-susceptible Dark Agouti (DA) rats. *Aia5* (= *Cia5*) from F344 rats on chromosome 10 also reduces AA severity [5]. Some of these loci also show differential gender influence, affecting either both sexes (*Aia1*), or only male (*Aia2*) or only female (*Aia3*). Three QTLs have been reported for oil-induced arthritis (*Oia1*, *Oia2* and *Oia3*) [3].

Susceptible strains of rats develop polyarthritis following injection of pristane (2,6,10,14-tetramethylpentadecane) [8,9]. Studies in the pristane-induced arthritis model have provided novel insights into the precise functional association of a genetic locus. In pristane-induced arthritis, the QTL *Pia4* has been narrowed down to one gene (*Ncf1*), which is part of the nicotinamide adenine dinucleotide phosphate oxidase complex [9]. The polymorphism of *Ncf1* leads to lower oxidative burst, and rats with this mutated gene develop more severe arthritis [9]. Increased production of reactive oxygen species (ROS) has also been implicated in the AA resistance of Albino Oxford (AO) rats [10].

Table 2

Factors affecting susceptibility/resistance to adjuvant arthritis

Effector pathways/response	Susceptibility/resistance (+/–)	Rat strains tested	References
MHC and non-MHC genes including particular quantitative trait loci influence arthritis susceptibility	+/–	Table 1	Table 1, [4,5]
Differential T-cell proliferative and/or cytokine response to Bhsp65, its arthritogenic epitope 180 to 188, or its regulatory C-terminal determinants	+/–	LEW/WKY/Wistar/F344	[20,24,30,31, 33,36]
Increased T-cell response to Rhsp65 and its regulatory epitopes	–	LEW/WKY	[20,24,26]
Anti-Bhsp65/Rhsp65 antibody response induced upon Mtb challenge	–	LEW/BN/WKY/F344/Wistar	[23,25,43]
Increased expression of hsp47 in the joints and enhanced anti-hsp47 antibodies in rats with arthritis	+	DA/AO	[45]
Immune response to hsp71	–	DA/AO/LEW	[45,46]
Migration into and retention within the target organ (joints) of arthritogenic leukocytes; the role of monocyte chemoattractant protein 1 and monocyte/macrophage chemotaxis	+	LEW/WKY	[19,20]
Increased reactive oxygen species contributing to arthritis resistance	–	DA/AO	[10]
Blunted hypothalamic–pituitary–adrenal axis activity	+	LEW/F344/Wistar	[51,53-55]
Microbial flora in a conventional housing environment	–	F344/Wistar	[56,59,60]

Bhsp65, mycobacterial heat-shock protein 65; hsp, heat-shock protein; MHC, major histocompatibility complex; Mtb, *Mycobacterium tuberculosis* H37Ra; Rhsp65, rat heat-shock protein 65.

Although the AA-resistant WKY rats have not been examined for arthritis-specific QTLs, studies on experimentally-induced autoimmune glomerulonephritis (GN) in the GN-susceptible WKY rats versus GN-resistant LEW and DA rats have unraveled several genetic loci controlling the disease susceptibility [11,12]. For example, the susceptibility of WKY rats to GN has been attributed in part to the low copy number of the gene *Fcgr3* compared with that in the GN-resistant LEW rats [11]. In the near future, this information might be relevant for evaluating the results of studies on WKY/LEW rats for AA susceptibility.

Studies in patients with RA have revealed genetic loci that have strong association with disease pathogenesis. These include, for example, the shared epitope, protein tyrosine phosphatase nonreceptor 22, signal transducer and activator of transcription 4, and tumor necrosis factor receptor-associated factor 1-complement component 5 [13-16].

Cell-mediated and humoral immune response to heat-shock proteins of arthritis-susceptible versus arthritis-resistant rat strains

AA can be induced by subcutaneous injection of LEW rats with Mtb in oil [1]. The paw inflammation starts after 8 to 10 days, peaks at about 15 to 16 days, and then undergoes spontaneous recovery in the subsequent 12 to 15 days following the arthritogenic challenge. The autoimmune inflammation of paws following Mtb injection is initiated by infiltration of mononuclear cells, mostly lymphocytes, macrophages and monocytes [1,17-20]. Several chemokines, including monocyte chemoattractant protein 1, play an important role in the pathogenesis of AA [18,19]. Monocyte chemoattractant protein 1 induces chemotaxis of monocytes into the joints, and it is expressed in the synovial tissue and blood when the clinical disease has progressed significantly [19]. Interestingly, inhibition of monocyte chemoattractant protein 1 after the onset of AA downmodulates the course of AA, highlighting the significance of the role of this chemokine in the progression of the disease [19]. For the T cells, the precise target antigen in AA is not clear, but the heat-shock protein hsp65 has been invoked in the disease process [21-24]. Unlike LEW rats, WKY rats are resistant to induction of AA following Mtb injection [24,25].

T-cell proliferative response to hsp65

Immune responses to mycobacterial heat-shock protein 65 (Bhsp65) play a critical role in the immunopathogenesis of AA in the LEW rat [21,22,24]. The T-cell response to Bhsp65 determinant 180 to 188 has been implicated in disease induction in LEW rats [21]. (Bhsp65 peptide 180 to 188 is cross-reactive with its longer version, Bhsp65 peptide 177–191 [24].) With progression of AA, arthritic LEW rats raise an immunoregulatory T-cell response to Bhsp65 C-terminal determinants that apparently contributes to recovery from acute AA [24]. Moreover, these rats spontaneously develop T-cell responses to (self) rat heat-shock protein 65 (Rhsp65) and the Rhsp65 C-terminal determinants, which are protective against AA [26,27]. The T cells against Bhsp65 C-terminal determinants are cross-reactive against Rhsp65 C-terminal determinants [27].

Generally, immune response to self-antigens is believed to initiate autoimmunity. Studies by other workers [28] and by us [26,29], however, have shown that immune reactivity to self-hsp65 is immunoregulatory in the AA model [26] as well as in patients with juvenile idiopathic arthritis [28]. Immunization of LEW rats with self-hsp65 affords protection against subsequent induction of AA by injection of Mtb in oil [26]. In juvenile idiopathic arthritis, increased immune response to self-hsp65 correlates with a favorable outcome of the disease [28].

We have compared the arthritis-susceptible LEW rats with the arthritis-resistant WKY rats that share the same MHC haplotype ($RT.1^l$) [20,24,30]. Following Mtb challenge, both LEW rats and WKY rats raised comparable levels of recall T-cell proliferative response to Bhsp65 and its arthritogenic determinant Bhsp65 peptide 177 to 191/Bhsp65 peptide 180 to 188 [30]. Unlike LEW rats, however, WKY rats raised a T-cell response to Bhsp65 C-terminal determinants early after Mtb injection. Moreover, WKY rats gave a higher level of recall T-cell response to Rhsp65 compared with that of LEW rats before the appearance of any clinical signs of AA in the latter [20]. These results suggest that the temporal kinetics of the appearance of a T-cell response against Rhsp65 and against Bhsp65 C-terminal determinants/Rhsp65 C-terminal determinants following Mtb injection is an important determinant of disease susceptibility in AA.

In a study on Wistar rats, a deficiency in T-cell response to Bhsp65 upon Mtb injection correlated with protection against AA [31]. Wistar rats without arthritis gave a significantly reduced level of T-cell response to Bhsp65 compared with rats with arthritis, but both rat groups gave a comparable level of T-cell response to the immunogen Mtb.

Unlike LEW rats, F344 rats are relatively resistant to AA. Differences in the T-cell repertoire against Bhsp65 and its arthritogenic determinant Bhsp65 peptide 180 to 188 have been proposed as one of the reasons for the AA resistance of F344 rats [32,33]. Furthermore, F344 rats were neither defective in antigen/epitope processing and presentation nor displayed active suppression of arthritogenic T-cells *in vitro* [33].

Cytokine responses induced by hsp65

New complexities regarding the role of cytokines in the immunopathogenesis of arthritis as well as other diseases are emerging as new information about cytokines comes forth [30,34-36]. AA was shown to be adoptively transferred to naïve rats by a well-characterized T-cell clone, A2b, which is a $CD4^+$ T cell that secretes IFNγ [21,37]. Similarly, Mtb-primed T cells that can transfer disease are mostly T-helper type 1 (Th1) [37]. Our studies in AA, however, have revealed a protective role of IFNγ and TNFα [30,36].

Until recently, most of the available information regarding the temporal cytokine profiles in arthritic LEW rats pertained to antigen nonspecific *ex vivo* cytokine secretion or to cytokine mRNA levels [38,39]. In our recent studies in AA [30,36], we observed higher Th1 cytokine levels against Bhsp65 during the recovery phase compared with those at the onset of or the peak phase of the disease. The cytokine response to Rhsp65 was found to be similar to that against Bhsp65 [20]. In general, the cytokine response of T cells against Bhsp65 and Rhsp65 as well as their defined antigenic determinants consisted predominantly of proinflammatory cytokines, IFNγ and TNFα. Furthermore, higher levels of proinflammatory cytokine secretion correlated with reduced severity of arthritis.

As AA is a Th1-mediated disease, this observation pointed to the role of Th1 cytokines in disease regulation. This inference indeed was supported by the cytokine profiles of Mtb-immunized, AA-resistant WKY rats. These rats produced high levels of Th1 cytokines early following Mtb injection, and the overall shape of the cytokine profiles was almost the opposite of that of LEW rats when followed against time post Mtb injection. Furthermore, treatment of LEW rats with TNFα or with Rhsp65 peptide 465 to 479, which primes a Th1 response, resulted in downmodulation of the clinical course of AA in LEW rats, albeit employing different mechanisms [30,36]. The levels as well as the timing of production of Th1 cytokines in response to Bhsp65/Rhsp65 therefore have a significant influence on the outcome of Mtb challenge of LEW/WKY rats. Other investigators have reported differences in the frequency/activity of T-helper type 2-cytokine secreting cells and $CD8^+$ suppressor/regulatory T cells in AA-susceptible rats (LEW rats, Holtzman rats) versus AA-resistant rats (BN rats, Buffalo rats, and Wistar Albino Glaxo rats) [40-42].

Two aspects of cytokine function are of significance when examining the susceptibility/resistance to AA. First, the proinflammatory cytokines (for example, TNFα and IFNγ) are not always pathogenic; instead, they can be immunoregulatory in nature [30,36]. Second, the T cells of arthritic LEW rats specific for the arthritogenic determinant of Bhsp65 (Bhsp65 peptide 180 to 188) produced IL-17 [30]. The contribution of IL-17 as well as the cytokines regulating IL-17 (for example, IFNγ, IL-4, and IL-27) in conferring susceptibility/resistance to AA, however, remains to be determined.

Antibody response to hsp65

AA can be adoptively transferred to naïve syngeneic recipient rats via the T cells but not the serum antibodies of arthritic rats. Antibodies *per se* are therefore not considered arthritogenic in AA. Studies by other workers and us [23,25], however, have revealed that antibodies against Bhsp65 and its mammalian homologue (Rhsp65) are protective in AA, and that the antibody profiles of AA-susceptible LEW rats are significantly different from that of AA-resistant BN rats and WKY rats.

In a study on Wistar rats immunized with Mtb, the nonarthritic subgroup of rats showed much higher antibody response

compared with that of the arthritic subgroup of rats – suggesting a protective role of anti-Bhsp65 antibodies [31]. In another study, it was shown that AA in LEW rats could be suppressed by a low dose of antibodies derived from the AA-resistant rat strains [43]. The AA-protective antibodies were reactive against mycobacteria and the depletion of anti-mycobacterial antibodies abrogated the protective effect of the antibodies. The protective antibodies were found in AA-resistant rats (F344 rats and BN rats) but not in AA-susceptible rats (LEW rats and Wistar rats). Furthermore, the presence or absence of these antibodies in different rat strains was not much influenced by the housing environment (germ-free versus conventional) in which the rats were kept [43]. In addition, serum transfer neither influenced the cell-mediated immune response to Mtb nor the protection against reinduction of AA in arthritis-recovered rats [43]. In contrast to the above results, another study indicated that natural antibodies to Bhsp65 are present in LEW rats but there was no association between the level of natural antibodies and the severity of AA [44].

Testing of the anti-hsp65 antibody response in naïve LEW rats, naïve BN rats and arthritic LEW rats revealed important differences in AA-susceptible rat strains versus AA-resistant rat strains [23]. Naïve LEW rats tested at different ages (6 weeks, 4 months and 9 months) showed spontaneous development of anti-Bhsp65 antibody response that gradually diversified in epitope reactivity. In contrast, naïve BN rats at an early age (6 weeks) showed reactivity to multiple epitopes of Bhsp65 that were also responded to by 9-month-old naïve LEW rats. Arthritic LEW rats showed reactivity to all these epitopes along with a couple of additional epitopes. The above three groups of rats also showed reactivity to two epitopes of self-hsp65, epitope 61 to 80 and epitope 436 to 455. Furthermore, pretreatment (tested separately) of naïve LEW rats with Bhsp65 peptide 31 to 46 and Bhsp65 peptide 37 to 52, Rhsp65 peptide 61 to 80, and antibodies to peptide 31 to 46 induced protection against subsequent AA [23].

In our study, both LEW rats and WKY rats showed antibody reactivity against multiple peptides of Bhsp65 and Rhsp65, but displayed opposite antibody profiles with time post Mtb injection [25]. The antibody response in WKY rats was broad to begin with but contracted with time post Mtb injection, whereas that of LEW rats was initially restricted to a few epitopes but later spread to include other epitopes. Eventually, the antibody response of both rat strains was narrowed down to the epitopes 31 to 46, 211 to 226 and 349 to 364 of Bhsp65 and to epitope 61 to 80 of Rhsp65. These epitope regions [25] are included among the epitopes reacted by sera of LEW rats and BN rats [23]. Moreover, adoptive transfer of sera of late phase arthritic LEW rats into naïve recipient LEW rats induced protection against AA [23,25]. There is therefore a common pattern and function of the humoral immune response to hsp65 shared by the arthritis-resistant WKY rats and BN rats, and this pattern in turn is different from that of arthritis-susceptible LEW rats.

Immune response to hsp47 and hsp70

In one study, the expression within the joints of two mammalian hsps (hsp47 and hsp72) of arthritis-susceptible DA rats was compared with that of arthritis-resistant AO rats [45]. Also examined was the relative T-cell proliferation and antibody response to hsp47 and mycobacterial hsp71. Important differences were revealed in these parameters in DA rats and AO rats following an arthritogenic challenge with Mtb.

Immunization with Mtb led to a significant increase in the expression of hsp47 in the joints of DA rats but not AO rats [45]. The expression of hsp47 presumably correlated with its role in procollagen production and processing. In contrast, no change in hsp72 expression in the joints was found in either rat strain, suggesting that the disease induction was not related to the local expression of hsp72. Both hsp47 and hsp71 caused an inhibition of lymph node cell (LNC) proliferation in AO rats but not in DA rats following *in vitro* restimulation of LNC with these hsps [45]. For the antibody response, an opposite pattern was observed for anti-hsp47 and anti-hsp71 antibodies [45]. The former were increased in DA rats, implying their association with a pathogenic response, while the latter were increased in AO rats, suggesting their link with protection against AA.

Another factor implicated in the AA resistance of AO rats is the increased production of ROS [10]. The ROS production was enhanced by hsp47 but not by hsp71, suggesting the role of hsp47 in controlling the disease process as well [10]. The protective effect of hsp71 in AA has also been reported in studies using the LEW rat [46]. hsp47 induces certain effector responses in the synovial tissue that facilitate disease induction/propagation, whereas the same protein may also contribute to downmodulation of the disease via increasing ROS [10,45,46]. The precise conditions (the level and timing of expression of hsp47) that lead to these two different outcomes need to be defined.

Cellular trafficking into the joints of AA-susceptible rats versus AA-resistant rats

Trafficking of arthritogenic leukocytes into the target organ is an integral component of the disease process in autoimmune arthritis. The synovial cellular infiltrate during the initial phase of inflammation in AA consists primarily of mononuclear cells (mostly monocytes, macrophages, and T cells) and relatively fewer neutrophils [1]. The arthritogenic T cells migrate into the synovium before appearance of the signs of clinical disease [38]. In our study, the adoptive transfer of ^{111}indium-labeled, Mtb-primed LNC of LEW rats into naïve recipient LEW rats resulted in the accumulation of arthritogenic LNC in the hind paws of the recipients within 20 hours, and the cellular migration gradually increased over 4 days [20]. Neither the transfer of Mtb-primed LNC of WKY rats into

LEW recipients nor the transfer of Mtb-primed LNC of LEW rats into WKY recipients resulted in retention of T cells in the hind paws of recipient rats.

Our findings in LEW rats are similar to those of other investigators who examined the migration of lymphoid cells derived from Mtb-primed or arthritis rats into the joints of syngeneic arthritis-susceptible (LEW/DA) recipient rats [47,48]. These investigators, however, compared arthritic rats versus control rats but not arthritis-susceptible rats versus arthritis-resistant rats. The migration of leukocytes into the joint involves their interaction with specific adhesion molecules (for example, E-selectin and very late antigen 4) [49] and chemokines (for example, regulated upon activation, normal T-cell expressed and secreted, and macrophage inflammatory protein 1α) and chemokine receptors (for example, chemokine (C–C motif) receptors 1 and 5) [50]. How the levels or the kinetics of surface expression of these cell-trafficking molecules influence arthritis susceptibility remains to be determined.

Hypothalamic–pituitary–adrenal axis activity in arthritis-susceptible rats versus arthritis-resistant rats

Studies in experimental arthritis models have shown that LEW rats, which are susceptible to AA as well as to streptococcal cell wall-induced arthritis, have reduced plasma levels of adrenocorticotropic hormone and corticosterone in response to streptococcal cell wall fragments as well as in response to IL-1α when compared with that of arthritis-resistant F344 rats [51]. The development and severity of arthritis in these two rat strains could be modulated in the opposite direction by administration of dexamethasone to LEW rats (which caused a reduction in arthritis severity) and by injection of a glucocorticoid receptor antagonist to F344 rats (which caused increased arthritis severity) [51].

A comparative study using electric tail shock to induce acute stress in five inbred rat strains, including LEW rats, F344 rats, and WKY rats – which were also subjected to altered (increased/attenuated) glucocorticoid negative feedback – showed that LEW rats displayed reduced response to stress (as assessed by measurement of adrenocorticotropic hormone and corticosterone) compared with F344 rats and WKY rats [52]. Furthermore, glucocorticoids can cause immune cells to undergo a Th1 to T-helper type 2 shift and increase the secretion of anti-inflammatory cytokines IL-10 and IL-4, while decreasing the secretion of proinflammatory cytokines TNFα and IL-1 [53]. This change in cytokine milieu, coupled with alteration in chemokines and chemokine receptor expression, can have a significant influence on the development and progression of arthritis, including AA, which is a Th1-mediated disease.

The involvement of the HPA axis in regulating peripheral inflammation was further validated by experiments showing that intracerebroventricular transplantation of fetal hypothalamic tissue from F344 rats to LEW rats led to a significant reduction in carrageenan-induced inflammation in recipient rats [54]. This transplant resulted in the expression of hypothalamic factors (for example, corticotropin-releasing hormone and corticosterone) in response to inflammatory stimuli.

With regard to behavior of rat strains and arthritis susceptibility, experiments in different rat strains (DA rats, LEW rats, AO rats, PVG rats, outbred Wistar rats) indicated that the development of arthritis and severity of the disease upon Mtb injection correlated well with specific behavioral patterns of the rat strains [55]. A study comparing apomorphine-susceptible versus apomorphine-unsusceptible Wistar rats showed that the former were relatively resistant to AA, whereas the latter developed arthritis of moderate severity [56]. In another study, Wistar rats were separated into two groups based on their learned helplessness (which also forms the basis for an animal model of depression), and were then immunized with Mtb [57]. Learned helplessness-negative rats developed AA earlier and of higher severity compared with learned helplessness-positive rats, although the corticosterone response to acute stress was much higher in learned helplessness-negative rats than that of learned helplessness-positive rats [57].

An interesting correlation has been observed between susceptibility/resistance of inbred rat strains to arthritis and drug addiction [58]. Rat strains that are highly susceptible to AA (for example, LEW rats and DA rats) are also prone to addiction to drugs like cocaine. Similarly, strains such as BN and F344 that are resistant to arthritis are also relatively resistant to addiction. As these two diseases/behavioral phenotypes also correlate with the level of HPA axis activity, common mechanisms underlying these three physiopathological parameters have been suggested [58].

Effect of gut commensal flora on susceptibility to autoimmune arthritis

Microbial agents, including environmental microbes and commensal bacteria in the gut, can modulate the severity of AA in certain rat strains. In studies using F344 rats, we [59] and other workers [32,60] have shown that exposure to environmental microbial agents can alter the AA susceptibility of this rat strain – in that F344 rats in a germ-free or barrier facility environment are susceptible to AA, whereas F344 rats raised in a conventional environment acquire resistance to AA.

In our study, barrier facility F344 rats were moderately susceptible to AA, whereas conventional F344 rats acquired resistance against AA. This AA resistance could be adoptively transferred to naïve barrier facility F344 rats via splenic T cells of conventional F344 rats, and it was attributable in part to the spontaneous generation of an anti-Bhsp65 T-cell response in conventional F344 rats but not in barrier facility F344 rats. This T-cell response was directed against the C-terminal determinants of Bhsp65, which are

also engaged in affording protection against AA in LEW rats [59]. Considering that hsp65 is a highly conserved protein, we suggested that the anti-Bhsp65 T-cell response observed in naïve conventional F344 rats is induced via determinant mimicry by hsp65-bearing environmental microbial agents in the conventional housing facility.

Additional mechanisms might also contribute to the acquired AA resistance in F344 rats, however, including tolerance induction in the subset of T cells directed against hsp65 and the pathogenic epitope of Bhsp65, bystander activation of T cells, effects of a superantigen, a differential HPA axis response at the onset of arthritis (with germ-free F344 rats but not conventional F344 rats showing an increase in plasma corticosterone) [56], and an alteration of the T-cell effector/T-cell regulatory balance by gut flora DNA [59,61]. It has been shown that interaction of gut flora DNA with TLR9 influences immune homeostasis in the intestine. TLR9-deficient mice have reduced Th1 and T-helper type 17 effectors, but an increased frequency of $CD4^+CD25^+$ regulatory T cells [61]. Moreover, gut flora DNA affects regulatory T-cell conversion *in vitro* by lamina propria dendritic cells [61]. The level and activity of $CD4^+CD25^+$ regulatory T cells can in turn modulate immunity to an infectious agent as well as an autoimmune disease.

Studies in Wistar rats have also revealed the suppressive effect of the conventional environment on AA [56]; however, no effect or a minimal effect of gut flora on arthritis development was reported in another study [62]. The relationship of autoimmune diseases, including RA, with gut microbial flora is a complex one – in that bacteria are considered to be a potential trigger for the initiation/ exacerbation of arthritis, while increased exposure to symbiotic microbes might ameliorate/prevent autoimmune diseases [63].

Conclusions

Multiple factors contribute to susceptibility/resistance of different rat strains to AA. Foremost among these is the MHC haplotype (for example, LEW rats ($RT.1^l$) versus BN rats ($RT.1^n$)). Rat strains having the same MHC haplotype (for example, LEW rats and WKY rats, both $RT.1^l$) but different non-MHC genes, however, may also differ significantly in their susceptibility to AA.

Specific QTLs are associated with susceptibility to AA. Interestingly, WKY rats are resistant to AA but are susceptible to GN [64]. A comparison of the mechanisms underlying the differential susceptibility of WKY rats to two different autoimmune diseases (AA and GN) might provide novel insights into genetic regulation of susceptibility to autoimmunity of human subjects with corresponding disorders.

LEW rats and WKY rats show an opposite temporal profile of cytokine response to Bhsp65/Rhsp65 when followed with time post Mtb injection. Surprisingly, despite AA being a Th1-mediated disease, proinflammatory cytokines play an important role in regulation of AA.

Antibodies to Bhsp65/Rhsp65 induced following Mtb challenge are protective against AA. Mtb-immunized LEW rats and BN (or WKY) rats reveal a differential antibody response to Bhsp65/Rhsp65, with diversification of response in LEW rats but a contraction of response in BN or WKY rats. Eventually, the antibody response in both rat strains becomes focused on specific epitopes of hsp65.

Mtb-primed leukocytes adoptively transferred into LEW rats and WKY rats show different kinetics of entry into and accumulation within the target organ (the joints), with an increased number of cells retained therein in LEW rats correlating with AA susceptibility.

Increased production of ROS is associated with resistance/ protection against AA as well as with other experimental arthritis, and oxidative burst-inducing drugs such as phytol can downmodulate acute phases as well as chronic phases of arthritis [8]. Phytol represents a novel category of therapeutic agents for the treatment of arthritis.

AA-susceptible rats (for example, LEW rats) differ from AA-resistant rats (for example, F344 rats and WKY rats) in their HPA axis activity in response to stress, with LEW rats displaying a defective HPA response.

F344 rats are highly sensitive to their housing environment – a conventional environment confers protection against AA, while a specific pathogen-free (or a barrier facility) environment is conducive to AA susceptibility.

Competing interests

The authors declare that they have no competing interests.

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