INFECTION AND AUTOIMMUNITY: LESSONS OF ANIMAL MODELS

M. Pásztói^{1,3*}, P. Misják^{1*}, B. György¹, B. Aradi¹, T. G. Szabó¹, B. Szántó¹, M. Cs. Holub¹,
Gy. Nagy^{1,2}, A. Falus^{1,3} and E. I. Buzás^{1**}

¹ Department of Genetics, Cell- and Immunobiology, Semmelweis University, Budapest, Hungary

² Department of Rheumatology, Semmelweis University, Budapest, Hungary

³ Research Group for Inflammation Biology and Immunogenomics, Hungarian Academy of Sciences, Budapest, Hungary

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While the key initiating processes that trigger human autoimmune diseases remain enigmatic, increasing evidences support the concept that microbial stimuli are among major environmental factors eliciting autoimmune diseases in genetically susceptible individuals.

Here, we present an overview of evidences obtained through various experimental models of autoimmunity for the role of microbial stimuli in disease development. Disease onset and severity have been compared in numerous models under conventional, specific-pathogen-free and germ-free conditions. The results of these experiments suggest that there is no uniform scheme that could describe the role played by infectious agents in the experimental models of autoimmunity. While some models are dependent, others prove to be completely independent of microbial stimuli. In line with the threshold hypothesis of autoimmune diseases, highly relevant genetic factors or microbial stimuli induce autoimmunity on their own, without requiring further factors. Importantly, recent evidences show that colonization of germ-free animals with certain members of the commensal flora [such as segmented filamentous bacteria (SFB)] may lead to autoimmunity. These data drive attention to the importance of the complex composition of gut flora in maintaining immune homeostasis. The intriguing observation obtained in autoimmune animal models that parasites often confer protection against autoimmune disease development may suggest new therapeutic perspectives of infectious agents in autoimmunity.

Keywords: autoimmunity, mimicry, infection, microbe, model

The concept of molecular mimicry

While recent progress in the field yielded several efficient new tools to reduce symptoms and to delay and/or prevent progression of some autoimmune diseases, the key causative/initiating events in disease development remain elusive. The observation that increased levels of disease-specific antibodies (such as anti-citrullinated protein antibodies or anti-DNA antibodies) may prevent disease onset by several years suggests that an early, unrecognized event may lead to slow progressive autoimmune disease development in genetically susceptible individuals.

Infections have long been considered as one of the possible causative factors that induce pathological autoimmunity. The suggested mechanisms include molecular mimicry and cross-reactivity based on structural similarity between microbial and host antigens, superantigen effect, upregulation of costimulatory and MHC (major histocompatibility complex) molecule expression, induction of cytokine and chemokine production, and modification of antigenic structures.

The term molecular mimicry was coined in 1965 [1]. The molecular mimicry concept gives an explanation of how similarity between foreign and self-antigens may lead to autoimmunity. Autoantigenic recognition of a given peptide has been shown to be related to structural propensities such as the extent of N-glycosylation [2], surface charge density, the presence of repetitive epitopes, surface-accessible position, relatively high mobility, and coiled coil character [3].

In our recent work, we compared known human autoantigenic sequences with non-autoantigen SwissProt entries with respect to bacterial–human exact sequence matches. We found that in the length range of immunologically relevant peptides (from the minimum of 8–9 aa for T-cell epitopes), exact sequence matches between human and bacterial proteins were found in the order of 10^5 . In support of the concept of molecular mimicry, we have shown that a larger proportion of known autoantigens contained at least 9-aa long linear exact human–bacterial matches compared to non-autoantigenic SwissProt/UniProtKB entries [2].

* These authors contributed equally.

**Corresponding author: Edit Buzas; Department of Genetics, Cell- and Immunobiology, Semmelweis University, H-1089 Budapest, Nagyvárad tér 4, Hungary; Phone: +36-30-7444918; E-mail: edit.buzas@gmail.com

Experimental models of autoimmunity

Inbred mice have proven to be extremely successful tools for basic immunology research. However, data argue for substantial differences between the immune systems of rodents and humans, and because of this, mice or rats have been recently considered of somewhat limited relevance as models of disease [4, 5]. However, despite all limitations, experimental models enable targeting fundamental questions such as the implication of infectious agents in autoimmune disease development.

Most human autoimmune diseases are characterized by complex genetics; a combination of numerous alleles confers genetic susceptibility for a given disease along with epigenetic and environmental factors. Exceptions include the rare monogenic autoimmune diseases such as the APECED (Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy) and IPEX (Immunodysregulation polyendocrinopathy enteropathy X-linked) syndromes in which the mutation of genes with outstanding impact on

immune tolerance (AIRE and FoxP3) leads to pathological autoimmunity. Experimental autoimmune models are in agreement with the above interpretation of the basis of human autoimmunity. They include two basic categories: induced and spontaneous ones (*Table 1*).

Induced models involve genetically susceptible strains of animals (in most cases mice or rats) that are injected with antigens in the presence of adjuvants. Induced models mimic human autoimmune diseases in which environmental stimuli trigger and modulate autoimmunity on a susceptible polygenic genetic background.

Spontaneous autoimmune models involve genetically manipulated transgenic strains of mice in which genetic alterations (affecting key molecules in immune tolerance and inflammation) lead to disease development spontaneously, without any evident environmental triggers. These latter models may be interpreted as murine counterparts of human monogenic autoimmune diseases. The strength of the spontaneous models is that they enable dissection of the role of single genes from other factors in autoimmune disease development.

Table 1. Experimental autoimmune models

Induced models	Spontaneous models
Collagen-induced arthritis (CIA) [44]	TNF α -transgenic arthritis [70]
Collagen-antibody-induced arthritis (CAIA) [45]	K/BxN arthritis [27, 48]
Zymosan-induced arthritis [17]	SKG arthritis [71]
Antigen-induced arthritis (AIA) [46]	Humanized mouse model of arthritis [72]
Streptococcal cell wall arthritis (SCWA) [10]	IL-1Ra KO mouse arthritis [73]
Adjuvant arthritis [6]	gp130R homozygous mutant arthritis [74]
Proteoglycan-induced arthritis (PGIA) [47]	DNase II-/-/IFN-IR-/- arthritis [75]
K/BxN serum transfer arthritis [27, 48]	HLA-B27 and human beta 2m transgenic rat [76]
Glucose-6-phosphate	MHC-deficient mouse arthritis [77]
Isomerase-induced arthritis [49, 50]	Non-obese diabetic (NOD) mouse [78]
MDP arthritis [7]	NOD.H2h4 thyroiditis [79]
Lyme arthritis [51]	Autoimmune diabetes in biobreeding (BB) rats [80]
T-cell hybridoma arthritis [52]	Scurfy mouse [81]
dsRNA arthritis [53]	HLA B27 tg uveitis model [22]
IX and X collagen arthritis [54]	MRL/lpr mouse lupus model [82]
COMP arthritis [55]	AIRE -/- mouse [83, 84]
Link protein arthritis [56]	TGF- β 1 KO SLE (systemic lupus erythematosus) [85]
Versican arthritis [57]	MLR/MP-lpr/lpr [86]
Mycoplasma arthritis [58]	NZB X NZW [87, 88]
<i>Staphylococcus aureus</i> arthritis [59]	MBP Tg mouse [89]
<i>Lactobacillus casei</i> arthritis [60]	Ulcerative colitis-like disease in mice with a disrupted interleukin-2 gene [90]
Pristane-induced arthritis and lupus [29, 61–63]	NZB/BL mice [91]
Experimental allergic encephalomyelitis (EAE, [20])	
Streptozotocin diabetes [64]	
Experimental autoimmune uveoretinitis (EAU) [65, 66]	
Autoimmune thyroiditis [67]	
Autoimmune inner ear disease in the rat [68]	
HTLV-1 tax Tg RA [69]

Fig. 1. The roles of infectious agents in experimental models of autoimmunity

In general, autoimmune animal models provide solid evidence that microbial substances are not only efficient modulators of autoimmunity but they are also fully capable of eliciting autoimmune inflammation. Importantly, they are essential components of adjuvants used at immunization in induced models. Furthermore, microbial products are efficient regulators of the incidence and severity of autoimmune symptoms (*Fig. 1*). The course and onset of autoimmunity can be compared in animals in conventional, specific-pathogen-free (spf), and germ-free housing conditions, and lessons of animal models may be translated to human autoimmune responses.

Microbial molecules may induce autoimmunity in animals

The vast majority of induced models involve the injection of exogenous or endogenous antigens with an adjuvant to induce chronic autoimmune disease development in genetically susceptible strains of mice or rats. Most immunizations involve injection of an antigen in an emulsion with complete Freund's adjuvant (CFA). CFA is composed of mineral oil containing inactivated and dried mycobacteria (usually *Mycobacterium tuberculosis*). The need for adjuvants to induce experimental autoimmune disease symptoms is explained by the slow release of antigens from the emulsion depots, and thus, by the prolonged presence of presented antigens. Microbial antigen-containing adjuvants (such as CFA) have been used successfully on the basis of empirical observations several decades before the discovery of danger receptors. Adjuvants provide pathogen-associated molecular pattern (PAMP) ligands that, in turn, induce expression of both MHC and costimulatory molecules of antigen presenting cells for efficient priming of naive T lymphocytes. It is of note that adjuvants by themselves have been shown to induce autoimmune diseases in rodents. This

is best exemplified by the classical adjuvant arthritis (AA) described in 1956 [6]. In this model, a progressive polyarthritis is induced by intradermal injection of rats by the oily emulsion of killed *M. tuberculosis* or *Mycobacterium butyricum*.

Muramyl dipeptide (MDP, *N*-acetylmuramyl-L-alanyl-D-isoglutamine), a peptidoglycan of mycobacterial cell wall, was also shown to induce polyarthritis upon injection to the footpad of rats [7]. In contrast, experimental allergic encephalomyelitis (EAE) in guinea pigs was shown to be suppressed by muramyl dipeptide [8], and immunosuppressive activity of muramyl dipeptide was also demonstrated in low-dose streptozotocin-induced (STZ) diabetes of mice [9].

There are several other examples for models in which microbial antigens induce autoimmune disease. Streptococcal cell-wall-induced arthritis (SCW) has been described in 1977. Intraperitoneal injection of cell wall preparations made of *Streptococcus A*, *B* and *C* results in the development of severe polyarthritis in female rats [10]. Injection of rats with *Borrelia burgdorferi* induces chronic polyarthritis (Lyme arthritis) [11]. Furthermore, *Mycoplasma arthritidis* T cell mitogen (MAM) as a superantigen has been shown to induce arthritis upon intraarticular injection to rats [12]. Interestingly, a single injection of a watery suspension of cell wall of *Lactobacillus casei* (a bacterium widely used for dairy production) induced dose-dependent, chronic, erosive, symmetric polyarthritis [13]. Furthermore, transient arthritis was induced by the intra-articular injection of lipopolysaccharide (LPS) in rats, rabbits, and pony [14]). *Staphylococcus aureus*-induced arthritis represents an infectious arthritis [15, 16]. Zymosan is an extract from yeast cell wall consisting of protein-carbohydrate complexes that induces chronic arthritis upon injection into the knee joints of mice [17]. Polymyositis, arthritis, and uveitis were found in a macaque experimentally infected with human T lymphotropic virus type I [18]. Endogenous retroviruses are implicated in the

pathogenesis of some human autoimmune diseases (such as SLE). Interestingly, highly increased expression of modified polytropic (mPT) env (envelope) RNA was recently observed in livers of lupus-prone mice as compared to non-autoimmune murine strains suggesting that lupus-prone mice may have a unique genetic mechanism responsible for the expression of mPT retroviruses [19].

Effect of microbial molecules on the course and severity of autoimmune diseases in various animal models

As summarized in Table 2, incidence and severity of symptoms of different experimental autoimmune models varied substantially depending on whether the animals were kept under conventional, spf or germ-free conditions. In some models, the absence of microbial stimuli led to reduced

symptoms (such as in the case of the EAE model). EAE is an animal model of multiple sclerosis induced by immunization of myelin basic protein (MBP) myelin oligodendrocyte glycoprotein (MOG) or proteolipid protein (PLP) or their peptides [20]. Under germ-free conditions, mice develop an attenuated EAE. Germ-free animals harboring segmented filamentous bacteria (SFB) developed EAE, showing that gut bacteria can affect neurological inflammation [21].

In HLA-B27 and human beta-2 microglobulin transgenic rats [22] under germ-free conditions, neither gastrointestinal [23] nor arthritis symptoms developed [24]. Similarly, in ankylosing enthesopathy (B10.BR(H-2k)) mouse (ANKENT) [25], the disease was prevented under germ-free conditions [26]. Furthermore, the widely used K/BxN arthritis [27] was also strongly attenuated [28] in the absence of microbial stimuli. Also, NZB mice had lower γ -globulin levels, higher frequency of serum antinuclear factor, and decreased incidence and severity of renal disease [29].

Table 2. Evidences obtained in various experimental models for the involvement of infectious agents

Conventional conditions	Spf conditions	Germ-free conditions	Augmentation by microbial products	Protection by microbial products
Collagen-induced arthritis (CIA) [44]		Enhanced disease [92]	Potentialiation by LPS [93]	Protection by <i>Ascaris suum</i> [21]
			Promotion of arthritis in mice by an antigen of <i>Mycoplasma fermentans</i> [94]	
Adjuvant arthritis (AA) [6]	Less severe arthritis [30]	100% incidence of arthritis [30]	Gram-positive bacteria (e.g. <i>Bifidobacterium</i> , <i>Propionibacterium acnes</i> and <i>Lactobacilli</i>) enhance the disease, possibly through their peptidoglycans [34]	Gram-negative bacteria (such as <i>E. coli</i> and <i>Bacteroides</i>) suppress the disease, possibly through their lipopolysaccharides [34]
Conventional F344 rats show significantly reduced incidence and severity [95]	Barrier-free (virus-free or specific-pathogen-free) F344 rats are susceptible to AA [95]			
HLA-B27 and human beta 2m transgenic rat [76]	Colitis and gastritis [23]	No gastrointestinal inflammation [23] No arthritis [24]	Obligate anaerobic bacteria, especially <i>Bacteroides</i> spp., may play a predominant role [23]	
Non-obese diabetic (NOD) mouse [78]	Spf NOD mice lacking MyD88 protein do not develop diabetes [32]	Germ-free MyD88-negative NOD mice develop robust diabetes [32]		Colonization of germ-free MyD88-negative NOD mice with bacteria (normally present in human gut) attenuates diabetes [32]
				Strong co-segregation of segmental filamentous bacterium positivity and diabetes protection in female mice [96]
Ankylosing enthesopathy B10.BR(H-2k) mouse (ANKENT) [25]	Disease develops [26]	No disease development [26]	<i>Bacteroides</i> spp., <i>Enterococcus</i> sp., <i>Veillonella</i> sp. and <i>Staphylococcus</i> sp., but not <i>Lactobacillus</i> sp., in germ-free mice [26]	

Table 2. (continued)

Conventional conditions	Spf conditions	Germ-free conditions	Augmentation by microbial products	Protection by microbial products
MRL/lpr mouse lupus model [82]		No difference at 5 months of age, possible contributory role for dietary exposure [97]		Improvement upon mycobacterial infection [98] Recovery after infection with IL-2/vaccinia virus [99]
K/BxN arthritis [27, 48]		Strongly attenuated [28]	Segmented filamentous bacteria (SFB) promote arthritis in germ-free mice [28]	
EAU [65, 66]			Staphylococcal enterotoxin B superantigen aggravates and induces relapse [100]	
SKG mice [71]			Triggered by beta glucan (Candida) [35]	
NOD aire ^{-/-} [33]		Is not prevented [33]		
Experimental allergic encephalomyelitis (EAE) [20]		Reduced symptoms [21]	SFB promote in germ-free mice [21], enhanced by concomitant administration of pertussis toxin (PTx); increased incidence and severity [102]	Intracerebroventricular PTx-mitigated symptoms [101] <i>Plasmodium chabaudi</i> suppresses the disease [36, 98] Repetitive PTx injections protect [96, 102]
Autoimmune inner ear disease in the rat [68]			Enhancement with PTx [68]	
Autoimmune thyroiditis in rats [67, 105]	Markedly reduced susceptibility [103]		Conventional intestinal contents re-established susceptibility [103]	
Pristane-induced lupus [29, 63]	Delayed onset and lower levels of autoantibodies [29], no disease			
MBP Tg EAE model [89]			Need for adjuvant plus PTx or PTx alone	
Conventional NZB mice [104]		Lower γ -globulin levels, higher frequency of serum antinuclear factor and decreased incidence and severity of renal disease [104]		

spf: specific pathogen free; LPS: lipopolysaccharide; PTx: Pertussis toxin

Interestingly, other models were developed with 100% incidence under germ-free conditions. These included collagen-induced arthritis (CIA) [28], adjuvant arthritis [30, 31], germ-free MyD88-negative non-obese diabetic (NOD) mice [32], NOD Aire ^{-/-} mice [33].

Numerous examples support that infectious agents are important modulators of experimental autoimmune disease severity and incidence. Several bacteria and bacterial products have been found to augment the diseases, while others are efficient in protection against the disease or attenuation of the symptoms (Table 2). The differential impact of spe-

cific bacterial infections has been clearly demonstrated in the EAE model in which Gram-positive bacteria (e.g. *Bifidobacterium*, *Propionibacterium acnes* and *Lactobacilli*) were shown to enhance the disease, while Gram-negative bacteria (such as *Escherichia coli* and *Bacteroides*) suppressed the autoimmune processes [34].

It is of note that molecules of not only bacterial but also fungal [35] or parasite origin [36] were demonstrated to modulate autoimmune models.

Intriguingly, some infectious agents proved to be protective against autoimmune diseases (Table 2). *Ascaris*

suum extract, introduced either *per os* or intraperitoneally, significantly and dose dependently inhibited cell influx and hypernociception in zymosan-induced arthritis, and reduced glycosaminoglycan loss and ameliorated synovitis. It also reduced intra-articular NO and IL-1 beta and IL-1 levels but not tumor necrosis factor in mice [37]. Mice subjected to *A. suum* extract had less severe CIA [37]. As we mentioned above, STZ diabetes and EAE have been shown to be reduced by MDP [8, 9].

Effect of microbial molecules via pattern recognition receptors [Toll like receptor (TLR) triggering]

Breakthrough discoveries in innate immunity during the past decades introduced danger receptors as key modulators of the immune response. TLRs participate in immune response to microbial pathogens through recognition of PAMPs, and they also sense host-derived ligands released upon tissue destruction.

Commonly used adjuvants (such as CFA) contain ligands that are highly efficient to stimulate TLRs.

In the IL-1 receptor antagonist knockout (IL1rn^{-/-}) arthritis model, the spontaneous onset was dependent on the bacterial flora. In the absence of TLR2 (in IL1rn^{-/-} Tlr2^{-/-} mice), arthritis developed in a more severe form, while the genetic absence of TLR4 in IL1rn^{-/-} mice provided protection against autoimmune arthritis. These data show that TLR2 and TLR4 play differential role in regulating immune response in this autoimmune model [38].

In Streptococcal cell-wall arthritis [10], the acute phase of inflammation was shown to depend on TLR2, whereas the chronic, erosive phase was characterized by the involvement of TLR4 coincident with the antigen-specific IL-17 response [39]. On the basis of the above observations, TLR4 appears to be a promising target in rheumatoid arthritis therapy. This is further supported by the recent finding that knockout of TLR4 was found to attenuate the pro-inflammatory state of diabetes [40].

The involvement of TLR signaling was investigated extensively in a retinal model of autoimmunity [41]. It was demonstrated that TLR2, TLR3, TLR4 and TLR9 were highly redundant in the adjuvant effect needed to induce experimental autoimmune uveitis (EAU), suggesting that diverse microbial infections may contribute to the pathogenesis of the disease. Moreover, the TLR3 ligand polyinosinic:polycytidylic acid (poly I:C) was shown to enhance the severity of EAU, pointing to the possibility that innate immunity mediated by TLR3 signaling may have an impact in the pathogenesis of virus-induced autoimmune diseases [42].

Furthermore, single-immunoglobulin interleukin-1 receptor-related molecule (SIGIRR/TIR-8), a known inhibitor of TLR signaling, was shown to regulate zymosan-induced and collagen-antibody-induced arthritides [43]. These data provide additional evidences in support of the role of TLR signaling in the development of autoimmune diseases.

Conclusion

The nature of the initiating events of autoimmune diseases has puzzled the scientific community for a long time. Among environmental factors possibly contributing to disease development in genetically susceptible individuals, infectious stimuli have been long hypothesized as major candidate triggers. In an effort to understand the impact of infections on autoimmunity, here we presented an overview of data obtained from various experimental models. The models not only enabled investigation of disease onset under SPF or germ-free conditions but also provided valuable tools to investigate the role of danger receptors (such as TLRs) signaling in the pathomechanism. Data obtained from experimental models show that in certain models, microbial stimuli are indispensable for disease development (such as in the case of the K/BxN model of arthritis), whereas other models do not depend on microbes (e.g. in NOD AIRE deficient mice). In line with the threshold concept of human autoimmunity, this may be explained by the differential impact of various genetic factors and environmental (such as microbial) stimuli to reach a disease threshold.

Recent evidences obtained from experimental autoimmune models point to the significance of SFB within the commensal flora. Colonization of germ-free animals solely by these bacteria may reestablish disease susceptibility, possibly through promotion of production of the proinflammatory IL-17.

Data summarized above strongly argue for an impact of microbial stimuli (including those of the commensal flora) on autoimmunity. The exploration of epigenetic changes that may take place in the immune system in parallel with different microbial stimuli remains an intriguing future field of research with implication to antibiotics consumption, probiotics food supplementation as well as autoimmune disease development.

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