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Genetic Heterogeneity of Autoimmune Disorders in the Nonobese Diabetic Mouse

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Abstract

The nonobese diabetic mouse is highly susceptible not only to diabetes but to several autoimmune diseases, and one might suspect that these are controlled by a shared set of genes. However, based on various gene-segregation experiments, it seems that only a few loci are shared and that each disorder is influenced also by a unique set of genes.

Introduction

Autoimmune disorders are regarded as chronic diseases in which immune responses are directed against self-antigens. The aetiology is unknown, but a genetic predisposition to autoimmunity has been observed. The first genes that came into focus in autoimmunity were the genes in the major histocompatibility complex (MHC) region based on their important functions in immune responses, and association with certain MHC haplotypes has been reported for most autoimmune diseases such as rheumatoid arthritis (RA), type I diabetes and systemic lupus erythematosus (SLE) [1–3]. However, besides the MHC region, it has been difficult to identify the loci associated with autoimmune diseases in humans. This is probably because of the complex nature of autoimmune diseases with several disease-modifying and interacting genes. Moreover, the possibility that various sets of alleles are of importance in different individuals adds to the complexity. Additionally, a broad range of environmental factors like diet, infections, toxic chemicals as well as age, gender and emotional stress are thought to affect the susceptibility to autoimmune diseases. In animal models, it is possible to limit the influence of all these factors. Obviously, animal models can never be directly comparable with the human diseases, but they provide a tool to study biological pathways involved in the pathogenesis of autoimmunity.

The nonobese diabetic (NOD) mouse strain spontaneously develops polyendocrine autoimmunity with chronic inflammation in several organ systems and is used as a model for type I diabetes, thyroiditis and Sjögren's syndrome [4–10]. The diabetes is the most well-characterized manifestation in the NOD mouse, and the first signs of inflammation in the islets of Langerhans (insulitis) are found at about 4 weeks of age. Nearly all NOD mice develop insulitis, but a substantial number of mice never proceed to the diabetic stage. In high-incidence colonies, 80–90% of the females and 40–50% of the males become diabetic after 3–7 months, because of extensive β-cell losses in the pancreas.

The insulitis precedes the development of inflammation of the salivary glands (sialadenitis), which is not apparent until the age of 8–12 weeks in females and >12 weeks in males. As in humans, the incidence of sialadenitis is higher in females than in males. The sialadenitis process seems not to be secondary to the diabetes development, as the MHC congenic strains NOD.B10.H2b and NOD.Q, which are protected from diabetes, develop sialadenitis [11–13]. Moreover, NOD is the only strain described that, like patients with Sjögren's syndrome, shows a decrease in tear and saliva flow rate because of the inflammation in submandibular and parotic glands [9].

In addition, a mild thyroid inflammation that is already evident at 1 week of age has been described in the NOD strain (I-A^{g7}), with an incidence ranging from a few per cent up to 80% [6]. Interestingly, the diabetes-resistant MHC congenic NOD strain, NOD.$H2^{h4}$ (I-A^{k}), shows an extensive infiltration in the thyroid gland, resembling Hashimoto's thyroiditis [14, 15], suggesting an important role for the MHC region in the disease development.

Although the tissue-specific response against, i.e. β-cells is the most prominent autoimmune response in NOD mice, they also develop systemic autoimmunity against a number of antigens in salivary glands, β-cells, thyroid, adrenal glands, testis, thymus and red blood cells. Moreover, a majority of aged NOD mice develop antinuclear antibodies (ANAs) and haemolytic anaemia [5].

Furthermore, the NOD mouse has been shown to be susceptible to induced autoimmune models such as experimental allergic encephalomyelitis (EAE) [16, 17], and it has also been used to study some features of lupus [18]. However, it is completely resistant to collagen-induced arthritis (CIA), a frequently used model for RA [19].

The aim of this review is to clarify the present knowledge of the genetic control of autoimmune disorders in the NOD strain. As the NOD mouse is subjected to several autoimmune diseases, one hypothesis has been that a certain set of alleles together with a complex interplay of various environmental factors could be responsible for many disease phenotypes. This is in line with the common gene hypothesis put forward by Becker, based on the identification of a set of gene clusters potentially involved in the development of a number of different autoimmune disorders [20, 21]. This is certainly relevant to the comparison of related autoimmune disorders like arthritis and encephalomyelitis [22, 23], but it is unclear whether genetic susceptibility to more diverse autoimmune diseases is shared. To address this question, the NOD mouse is a particularly interesting model because of the coexistence of different types of autoimmune diseases such as diabetes, sialadenitis and encephalomyelitis. Based on the data presented by various gene-segregation experiments published so far, it seems, however, that each of the autoimmune disorders of the NOD mouse is mainly controlled by unique sets of disease-promoting alleles (Figs 1 and 2), but that some disorders are more similar than others.

Genetic control of autoimmune disorders in the NOD mouse

Type I diabetes

The genetic contribution to type I diabetes in the NOD mouse has been extensively studied, and several loci contributing to the development of diabetes or insulitis have been identified in crosses involving the diabetes-resistant C57Bl/6 (B6) or C57Bl/10 (B10) (Fig. 2). *Idd1* located in the MHC region on chromosome 17 is a major locus for diabetes [12, 24]. The $H2^{g7}$ haplotype of the NOD mouse promotes the development of diabetes, whereas other haplotypes have a dominant protective effect. The various penetrances of diabetes compared with sialadenitis or thyroiditis in NOD ($I\text{-}A^{g7}$), NOD.Q ($I\text{-}A^{q}$), NOD.B10.H2b ($I\text{-}A^{b}$) and NOD.$H2^{h}4$ ($I\text{-}A^{k7}$) suggest an important genetic difference affecting the presentation of autoantigens by the MHC class II molecules in these diseases.

In addition to the MHC region, several other diabetes-associated loci have been identified (Fig. 2), and several of these loci have been confirmed to affect the diabetes development using congenic strains. Besides the MHC region, the *Idd3* locus mapped to a <1 cM interval on chromosome 3 shows the strongest influence on diabetes this far, as the disease-resistant B6 allele could reduce the disease by 70% in females and by 95% in males on the NOD background [25, 26]. The underlying genes for diabetes have not yet been determined, but the tightly linked *Il2* gene on chromosome 3 [27], the genes encoding the costimulatory molecules CTLA-4 and CD28 on chromosome 1 [28] and CD30, TNFR2 and CD137 on chromosome 4 [29] have been proposed to be important. These genes encode proteins involved in T-cell expansion and activation, and there is strong evidence that T-cell-mediated pathways are important for the development of diabetes.

Moreover, the complexity of diabetes in the NOD mouse was clearly demonstrated by the observation that combinations of *Idd* loci, that on their own showed minor or no protection, provided a nearly complete resistance [29–31].

Sialadenitis

The genetic basis of sialadenitis is largely unknown. Associations of Sjögren's syndrome with certain MHC haplotypes are observed in humans, but no clear evidence for the MHC region has been demonstrated in animal models. However, a genetic component of sialadenitis is evident, as only a few strains develop the disease spontaneously.

Two separate gene-segregation experiments have recently been performed in crosses with NOD and B6 or B10 with different results [13, 32]. Boulard *et al.* identified one locus on chromosome 1 linked to sialadenitis in both sexes and one locus on chromosome 3 linked to sialadenitis in females [32] (Fig. 2). These two loci overlap with the two diabetes loci, *Idd5* and *Idd3*, respectively, which have earlier been suggested to contribute to some sialadenitis-associated phenotypes from the analyses of congenic strains [33]. Recently, double congenic B6 mice carrying two NOD fragments, one covering *Idd5* and one covering *Idd3* and most likely *Idd10* and *Idd17*, have been reported to have most of the Sjögren's syndrome-associated phenotypes found in the NOD strain, such as decreased saliva and lacrimal flow rate, increased salivary protein content, decline in amylase activity and focal lymphocytic infiltrations in submandibular glands [34].

In contrast to Boulard *et al.*, we did not identify the *Idd3* and *Idd5* regions as being linked to the sialadenitis in our (NOD.Q × B10.Q)F2 cross. Instead, a locus on chromosome 4, *Nss1*, which was associated with the severity of sialadenitis, was found [13]. No *Idd* loci are located in the

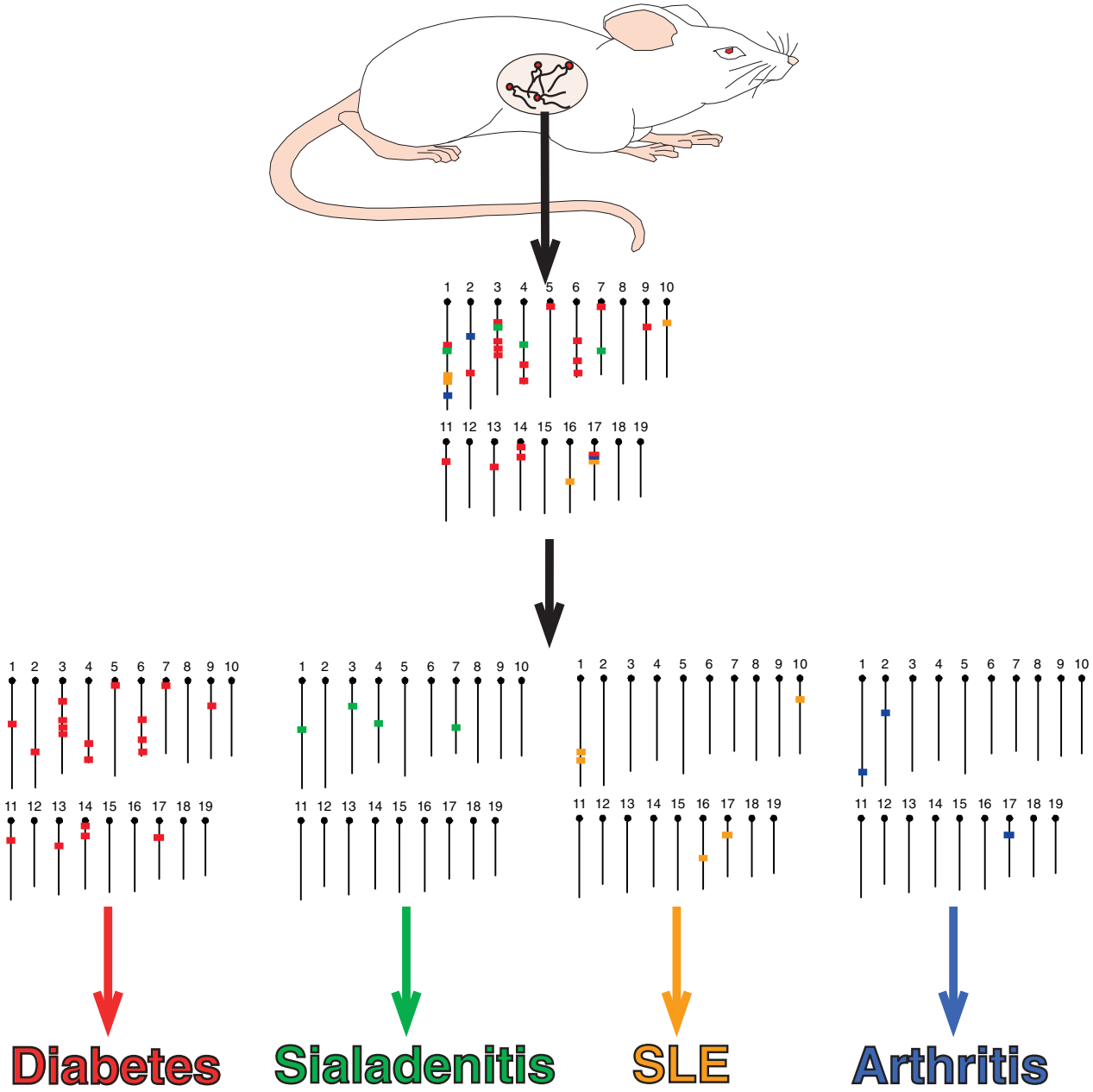

Figure 1 Genetic heterogeneity of autoimmune disorders in the nonobese diabetic (NOD) mouse. The chromosomal maps show the location of reported quantitative trait loci associated with type I diabetes, sialadenitis, systemic lupus erythematosus (SLE) or arthritis in the NOD mouse [13, 19, 24, 31, 38, 42, 71, 74–81]. Type I diabetes loci are indicated in red, loci associated with sialadenitis in green, SLE-associated loci in orange and arthritis loci in blue. Chromosomal positions are based on the map from the Jackson Laboratory (http://www.informatics.jax.org).

region that contains *Nss1*, thus indicating that this is a unique sialadenitis locus in the NOD mouse in comparison with diabetes. The different results obtained in the two gene-segregation experiments could be because of different experimental set-ups. We studied the association of arthritis with sialadenitis after the induction of CIA, whereas Boulard *et al.* used unimmunized mice, and an effect of the arthritis induction on the sialadenitis phenotype could not be excluded. Moreover, the definition of sialadenitis was slightly different; Boulard *et al.* defined an inflammatory focus as the accumulation of 10 mononuclear cells, whereas we put the lower limit to 50 mononuclear cells. Furthermore, the age of the mice differed in these two studies, and as the incidence of sialadenitis increases with age in the NOD mouse, an effect on the linkage analysis results is possible.

In addition, another unique sialadenitis locus in the NOD mouse has been identified on chromosome 7 in

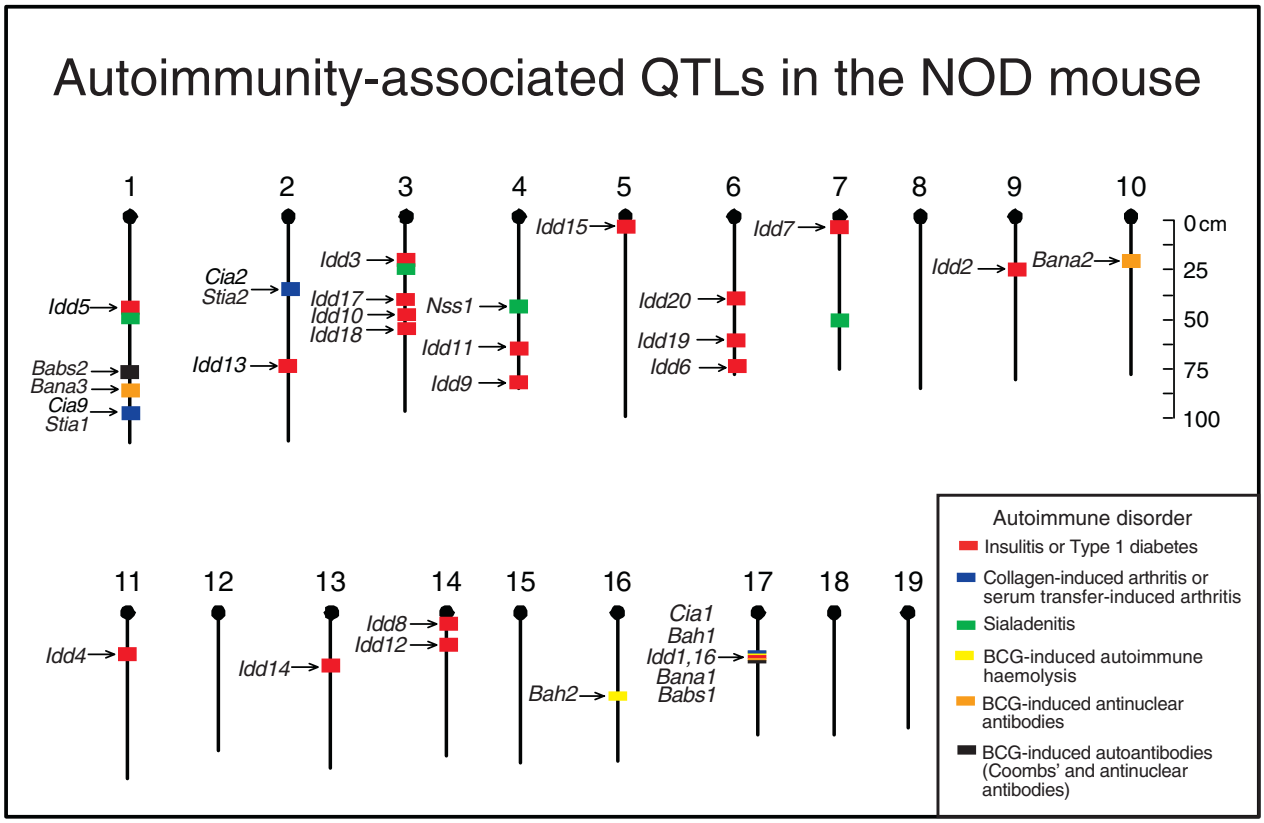

Figure 2 Quantitative trait loci (QTLs) identified in crosses with the nonobese diabetic (NOD) strain. The chromosomal map shows the location of autoimmunity-associated QTLs identified in the NOD mouse [13, 19, 24, 31, 37, 41, 70, 73–80]. Chromosomal positions are based on the map from the Jackson Laboratory (http://www.informatics.jax.org).

males from an (NOD × NZW)F2 cross [32]. Even though the genetic control of sialadenitis and diabetes is overlapping in a few cases, there are unique sialadenitis loci present, indicating that the genetic control of the disease is separated from that of diabetes. The linkage to the MHC haplotype $H2^{g7}$ is one of the strongest diabetes-associated loci in the NOD mouse, but interestingly, there is no published evidence for an influence of the MHC region on the development of sialadenitis in the NOD.

Collagen-induced arthritis

CIA is induced by intradermal injections of type II collagen, a major component of joint cartilage, together with an adjuvant and resembles RA in many clinical, histological and genetic aspects [35]. The genetic contributions to CIA susceptibility have been investigated during a number of years, and crosses between resistant and susceptible inbred strains have demonstrated that CIA is a complex polygenic trait. As in humans, the MHC-encoding genes are important for arthritis development in mice [35], as CIA is mainly associated with the $H2^{q}$ and $H2^{r}$ haplotypes. However, several other genetic loci have been reported as being linked to CIA susceptibility (reviewed in [36]). To investigate whether NOD genes also promote autoimmune-mediated arthritis, we established an NOD strain with an MHC class II fragment containing the A^{q} class II gene predisposing to CIA (NOD.Q). Surprisingly, this mouse was resistant to arthritis in contrast to other A^{q}-expressing strains such as C57Bl/10.Q (B10.Q) and DBA/1. However, we recently identified a NOD locus on chromosome 1, *Cia9*, in an F2 cross with NOD.Q and B10.Q strains that enhanced arthritis severity in CIA (Fig. 2). Interestingly, another NOD locus, *Stia1*, overlapping with *Cia9* has been shown to confer susceptibility to serum transfer-induced arthritis in an (NOD × B6)F2 cross [37] (Fig. 2). Furthermore, an arthritis-protective NOD allele on chromosome 2 was identified through gene-mapping experiments both in the CIA model and in the serum-transfer model (Fig. 2) [19, 37, 38]. These arthritis loci are unique in comparison with the published *Idd* loci, suggesting a different genetic control of arthritis in comparison with diabetes (Fig. 1).

SLE-associated phenotypes

SLE is a systemic autoimmune disease characterized by the presence of pathogenic antibodies to a variety of

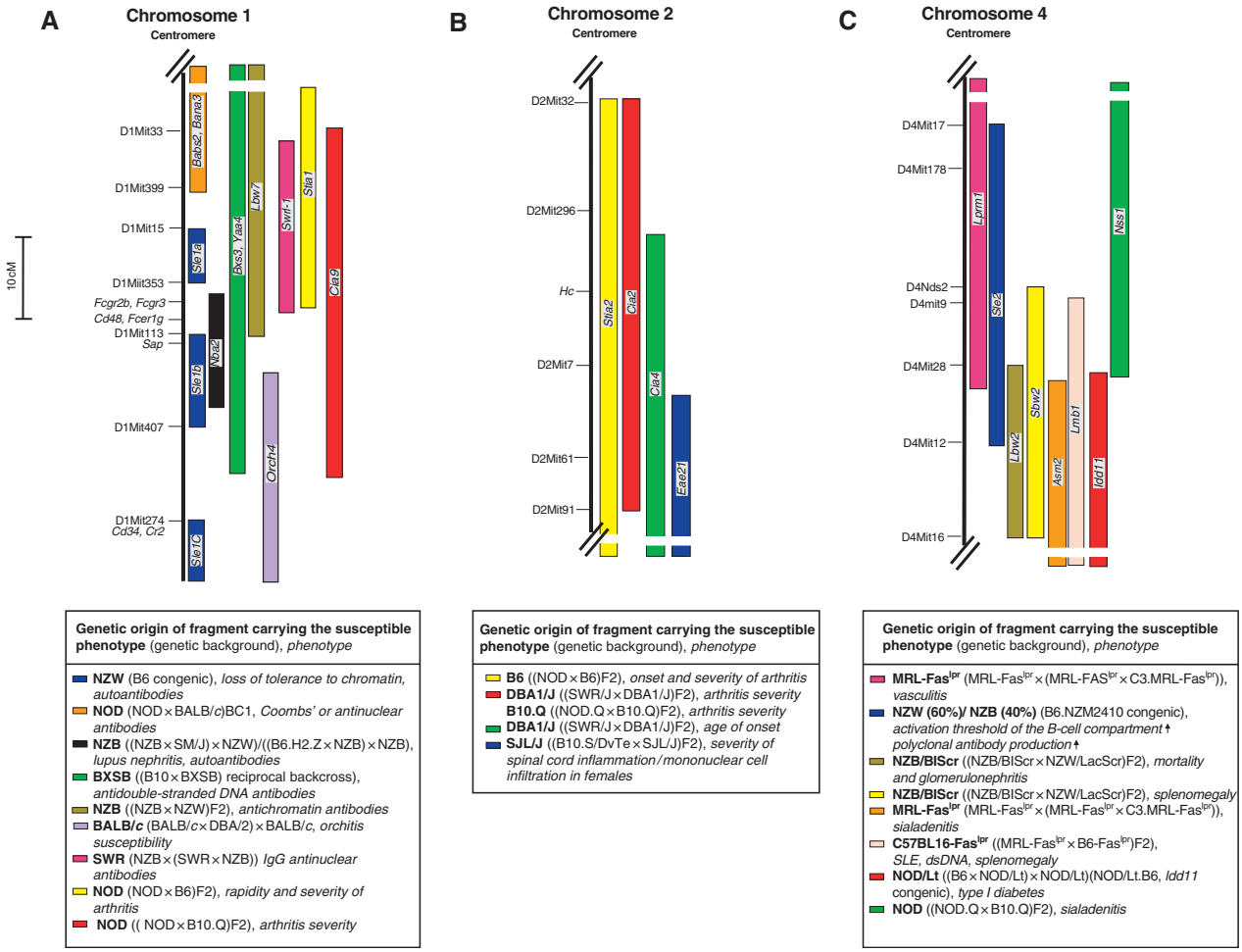

Figure 3 Colocalization of non-*Idd* loci on chromosomes 1, 2 and 4 in the nonobese diabetic (NOD) strain with quantitative trait loci (QTLs) associated with autoimmune disorders in other strains. The figure shows the colocalization of non-*Idd* loci in the NOD strain with QTLs associated with autoimmune disorders in other strains in selected parts of (A) chromosome 1 [19, 37, 41, 43–45, 47–49], (B) chromosome 2 [19, 37, 56, 81] and (C) chromosome 4 [13, 43, 63–65, 69, 71]. Chromosomal positions are based on the map from the Jackson Laboratory (http://www.informatics.jax.org).

self-proteins, in particular nuclear antigens. It is clinically manifested by unpredictable exacerbations and remissions of manifestations from several organ systems such as kidneys, skin, joints, lungs, brain and heart.

Systemic autoimmunity in the mouse has been used as a model for human SLE for a considerable amount of time. The three main lupus-prone strains are: the (NZB × NZW)F1 and the related NZM2410 recombinant strain; the MRL/*lpr* strain carrying the *lpr* spontaneous mutation in the FAS receptor gene; and the BXSB strain carrying the Y chromosome autoimmune accelerator (*yaa*) gene. All these strains develop spontaneous systemic autoimmune disease, with several features resembling the human SLE [39, 40].

The NOD mouse spontaneously develops some features of SLE with age such as ANA and haemolytic anaemia [5]. However, the systemic autoimmunity in NOD mice appears at earlier age and is markedly increased when treating with *Mycobacterium bovis* (bacille Calmette–Guérin (BCG)). Administration of heat-killed BCG to prediabetic NOD mice prevents the development of type I diabetes. It was discovered, as a result of the investigations of the mechanisms of BCG treatment of the diabetes in NOD mouse, that BCG instead induces a lupus-like disease characterized by mild focal nephritis, haemolytic anaemia and ANA directed against double-stranded DNA and Smith/ribonucleoprotein complex, as well as increased severity of sialadenitis [18]. This is one of the few models for autoimmune diseases, where the environmental trigger is known. And, as the BCG treatment does not seem to induce ANA production in other strains [18], the genetic background of the NOD mouse plays a role in the aetiology of the induced lupus-like disease. Therefore, using the BCG-induced lupus model in NOD mice, Jordan *et al.* [41] performed gene-segregation experiments in NOD × (NOD × BALB/*c*)-backcrossed mice to investigate the genetic susceptibility to lupus in NOD mice in the light of the common gene hypothesis [20, 21, 42].

Surprisingly, the only gene region that influenced the development of both diabetes and the autoantibody production of BCG-induced lupus in NOD mouse was the *H2* region. This indicates that the environmental agent, BCG, triggers distinct pathways inducing systemic autoimmune response, overriding the tissue specificity of the type 1 diabetes. In addition to the *H2* region, three gene regions were identified on chromosomes 1, 10 and 16, controlling haemolytic anaemia, ANA or autoantibodies (ANA or Coombs') (Fig. 2). The region on chromosome 1, found to control autoantibody production, has previously been implicated as susceptibility loci for ANA production and nephritis in other lupus models (Fig. 3A) [43–45]. No gene region of significant linkage was found controlling glomerular nephritis in the cross.

In addition to the BCG-induced lupus, a similar disease may be induced in NOD mice by transferring human anti-DNA monoclonal antibodies carrying the pathogenic 16/6 idiotype [46]. Just as the BCG treatment, the 16/6 idiotype treatment resulted in a significant reduction in diabetes incidence (25% versus 90%). This model again points towards the fact that a change in induction or regulation of the immune system in the NOD mouse may cause a shift in autoimmune response, resulting in different autoimmune diseases. The situation in NOD mouse may be applicable to the human situation, where more than one autoimmune disease may segregate in a family.

Colocalization of loci associated with autoimmunity – importance of certain pathways involved in the pathologic process

As mentioned already, several gene-segregation experiments have been reported using the NOD strain (Figs 1 and 2), and the genetically best-characterized disease is type I diabetes. Surprisingly, most of the other disease manifestations in the NOD mouse seem to be under a unique genetic control in comparison with diabetes, even though a few regions are shared, such as the MHC (Figs 1 and 2). Perhaps, that could be explained by the use of different pathogenic pathways leading to disease in diabetes, compared with the other disorders. Below, we go through and discuss the importance of some disease pathways that seem to be vital for disease development in recently described non-*Idd* loci on chromosomes 1, 2 and 4 in the NOD mouse and compare them with non-NOD-associated loci located in the same positions.

Antibody-associated autoimmune disorders are linked to the distal part of chromosome 1

Several phenotypes associated with autoantibodies are linked to the distal part of chromosome 1 in the NOD mouse as well as in other strains, with related disorders as seen in Fig. 3A, indicating the importance of this region in antibody-dependent autoimmune disorders [41, 43–45, 47, 48]. *Cia9*, *Orch4* and *Stia1* are not directly associated with autoantibody phenotypes but rather with the overall disease incidence or severity in each model [19, 37, 49]. However, in serum-transferred arthritis, autoantibodies against glucose 6-phosphate isomerase provoke the arthritis [38], and in CIA, autoantibodies play an important role in the pathogenesis of arthritis [50]. Moreover, autoantibodies against testicular antigens have been identified in the development of orchitis [51]. Interestingly, no diabetes loci are located in this region (Fig. 2), and perhaps that could be because of a minor role of autoantibodies in the pathogenesis of diabetes.

The colocalization of genetic loci associated with the production of autoantibodies as well as with susceptibility to antibody-dependent diseases indicates that the distal part of chromosome 1 harbours genes either important for autoantibody formation or associated with the effector phase of pathogenic antibodies. The linked fragment is big, and most likely it contains several genes or gene clusters of importance for the development of the various autoimmune disorders.

The most carefully characterized locus on distal chromosome 1 is *Sle1*, which is associated with the breakdown of tolerance to chromatin, preferentially the H2A/H2B/DNA nucleosomes. The *Sle1* locus has been further subdivided into three separate loci, *Sle1a*, *Sle1b* and *Sle1c*, and each of them independently affects the loss of tolerance to chromatin [48].

The complement receptor (CR) 1- and 2-encoding gene, *Cr-2*, has been proposed as a candidate gene for *Sle1c* [52]. CR1 and CR2 are expressed on the surface of follicular dendritic cells and B cells and bind complement factor 3 or 4 that is covalently bound to antigens or immune complexes. Furthermore, CR1 and CR2 have been implicated in the pathogenesis of SLE, as patients with SLE have approximately 50% lower levels of CR1 and CR2 on B cells.

The Fc receptor (FcR) gene cluster, containing the inhibitory *Fcgr2b*, the immunoglobulin G immune complex binding *Fcgr3* and *FcerIg* genes, is highly polymorphic and has been postulated to play a role in autoantibody-mediated autoimmune diseases, although concluding data are missing. A deletion of the *Fcgr2b* backcrossed into the DBA/1 mouse led to a more severe CIA [53], whereas a deletion of the *Fcgr3* protected the mice from CIA [54]. The same effects were observed using a model in which arthritis was induced with anti-CII antibodies (Nanda Kumar *et al.*, in preparation). Interestingly, based on both knockout and congenic experiments, the FcR gene cluster has been excluded as candidate genes for the *Stia1* locus in serum-transferred arthritis [37], suggesting that only transfer with anti-CII antibodies is associated with FcR polymorphism. Moreover, the FcR gene cluster,

which is closely linked to *Sle1a* and *Sle1b*, has been excluded as a major candidate for these loci [47], although it may operate as an epistatic modifier to a gene within *Sle1* [55].

Could C5 deficiency mediate protection against arthritis?

Resistance to arthritis mediated by NOD alleles has been linked to the proximal part of chromosome 2 in two separate models of arthritis, the CIA model (*Cia2*) and the serum transfer-induced model (*Stia2*), as seen in Fig. 3B [19, 37, 56]. In addition, a SWR/J allele mapped to the same region showed a protective effect in gene-segregation experiments involving (DBA/1 × SWR/J)F2 crosses (Fig. 3B) [56–58]. Furthermore, the rat *Cia11* locus, identified in an F2 cross with DA and NB rats, is homologous with *Cia2* [59], and thereby further strengthens the importance of this region in modulating CIA. Interestingly, both NOD and SWR/J mice are naturally deficient in C5, through gene deletions, and the arthritis-protective alleles derived from NOD and SWR/J mice cover the location of the C5-encoding gene (*Hc*). The lack of C5 in the NOD mouse seems not to affect the development of diabetes, as no known *Idd* loci are linked to this region (Fig. 2), indicating that arthritis and diabetes are controlled by different alleles and most likely use various pathogenic pathways.

The importance of C5 in arthritis development was supported by the findings that DBA/1 mice lacking C5, through targeted gene deletion, were relatively resistant to CIA [60], and that treatment with anti-C5-neutralizing antibodies efficiently blocked the development of arthritis [61]. However, this does not necessarily mean that C5 is an absolute requirement for arthritis. We have recently described that a few C5-deficient mice in an (NOD.Q × B10.Q)F2 cross developed arthritis [19]. This is in accordance with an earlier finding in a cross involving the highly susceptible DBA/1 strain and the resistant SWR/J strain [62]. In addition, congenic mice, carrying one NOD allele and one B10 allele at the *Cia2* locus, were protected against arthritis, even though they had normal serum levels of C5 [19]. This protective effect in heterozygous mice could be explained by a decreased C5 production in the inflamed tissue, which is difficult to measure in the serum. Alternatively, there is another linked gene that modifies the development of arthritis.

A cluster of immune-regulating genes on chromosome 4?

As has already been discussed in the present review, certain regions in the genome seem to harbour genes or clusters of genes controlling autoimmune pathways. One such region may be located on chromosome 4. In this region, several loci have been mapped that are involved in the development of systemic autoimmune disease, in particular features of lupus, as shown in Fig. 3C: *Sbw2* and *Lbw2* [43], *Lprm1* [63], *Lmb1* [64], *Sle2* [65], *Nba1* [66] and *Lmh1* [67]. There may even be at least three distinct lupus clusters, although there is still not enough fine mapping information to draw that conclusion. It should also be mentioned that two loci involved in the development of autoimmune gastritis have been located on chromosome 4, *Gasa1* and *Gasa2* [68].

Three loci originating from NOD have been located on the same chromosome – the telomeric *Idd11* [69] and *Idd9* [70] and the centrally located sialadenitis locus *Nss1* [13]. The diabetes and sialadenitis in NOD mouse is probably driven by different pathways, as has been shown in the BCG treatment of NOD mouse. This is also supported by the probable nonoverlap by the *Idd* and *Nss* loci in the region.

An additional sialadenitis locus has been located on chromosome 4 – *Asm2* (Fig. 3C) [71]. *Asm2* was identified in a cross with MRL/lpr mice. It should be emphasized that the sialadenitis in the MRL and NOD strains are most likely two different diseases and thereby partially controlled by different sets of genes [72]. Interestingly, the *Asm2* region overlaps with *Idd11*.

Concluding remarks

The suggestion that autoimmune disorders may be controlled by a common set of susceptibility genes/gene clusters is known as the common gene hypothesis [42]. To certain extent, this seems to be the case for experimental models of arthritis and encephalomyelitis. In a comparison of gene-mapping studies of arthritis and encephalomyelitis in mouse and rat, approximately 50% of the identified gene regions were shared between the disease models when using similar strains [23] (Fig. 4). Notably, there is also a clear genetic correspondence between species, including mouse, rat and human, for these diseases. However, when increasing the spectra of autoimmune diseases to include disorders such as type I diabetes, SLE and sialadenitis, the picture of shared genetic regions does not hold to the same extent as seen in Fig. 4. It is most relevant to believe that the pathogenesis of these diseases is more divergently controlled.

On the other hand, there are some aspects of autoimmunity, such as inflammatory effects caused by autoantibodies, which are influencing many different autoimmune disorders. This has become apparent by the colocalization of the loci associated with the production of autoantibodies or with other autoantibody-related phenotypes in several disease models to the telomeric part of mouse chromosome 1 (Figs 3A and 4). It is, however, not likely that a single gene controls this autoantibody effect. Rather, the work by Wakeland *et al.* on the *Sle1* locus clearly points towards a cluster of several genes, all influencing

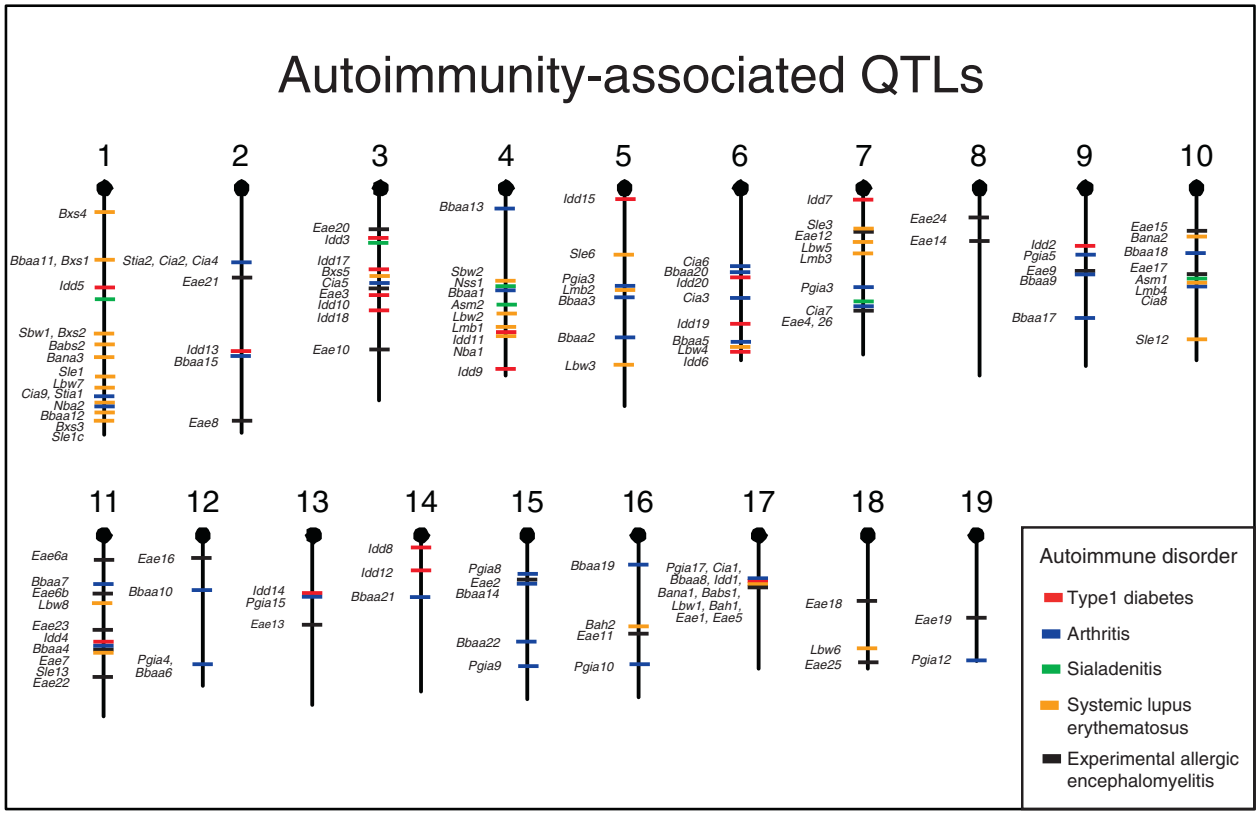

Figure 4 Map of Quantitative trait loci (QTLs) identified in various autoimmune disorders. A chromosomal map of QTLs associated with diabetes, experimental allergic encephalomyelitis, various forms of arthritis, sialadenitis or with systemic lupus erythematosus. These loci are identified in many different crosses of mice [13, 19, 24, 31, 35, 37, 41, 43, 47, 48, 56, 64, 66, 70, 71, 73–100], and the map shows the approximate locations as several of these loci cover several centimorgans. Chromosomal positions are based on the map from the Jackson Laboratory (http://www.informatics.jax.org).

the loss of tolerance to nuclear antigens. And, as more and more disease-associated loci dissolve, the situation of genes of similar function segregating as clusters is often seen.

The NOD is in many aspects an autoimmune-prone strain. It spontaneously develops several autoimmune tissue-specific diseases, and it is also susceptible to induced diseases. The coexistence of several autoimmune disorders in the NOD strain opens the possibility of investigating to what extent these diseases are controlled by a shared set of alleles. An earlier comparison between genetic loci associated with arthritis and encephalomyelitis suggested a relatively high overlap between the different diseases [23]. The gene-segregation experiments published to date indicate, however, that the genetic control is more distinct for each autoimmune disorder in the NOD mouse.

The use of different pathogenic pathways, in the various diseases, in the NOD mouse, is the most obvious explanation for the unique genetic control of each disorder. The MHC haplotype, for example, seems to be vital for diabetes, arthritis and encephalomyelitis development, whereas no major influence of MHC has been reported for sialadenitis development, and there is no clear evidence for the role of MHC class II genes in the development of lupus (Fig. 4).

Notably, the use of the different autoimmune pathways in the NOD mouse can in some, but not all, cases depend on the initial events triggering the immune system. 'External' disease triggers, such as the introduction of an adjuvant, can provoke a different autoimmune response than 'internal factors', i.e. the status of inhabitant microorganisms, sex hormones and stress, that are important for the development of spontaneous diseases.

Interestingly, however, within the NOD strain, there seem to be genes or clusters of genes that are involved in pathways shared between autoimmune diseases of different types. Such examples are the regions of *Idd3* and *Idd5* on chromosomes 1 and 3, respectively. These loci have been suggested to contribute to the development of both diabetes and sialadenitis.

It is important to remember that the search for genes associated with autoimmunity is still in its infancy. New quantitative trait loci (QTLs) are to be found, and many of the identified QTLs need to be confirmed in congenic strains, sequenced and cloned. The identification of the different disease-associated genes localized in crosses with NOD mice will unravel the autoimmune disposition of this strain and increase our knowledge on the difference in tissue-specific and systemic autoimmune diseases.

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