Journal of Autoimmunity 58 (2015) 90–99

Contents lists available at ScienceDirect

Journal of Autoimmunity

journal homepage: www.elsevier.com/locate/jautimm

Autoimmunity and antibody affinity maturation are modulated by genetic variants on mouse chromosome 12

Roxanne Collin [a, b], Véronique Dugas [a, b, c], Geneviève Chabot-Roy [a], David Salem [d], Astrid Zahn [e], Javier M. Di Noia [e, f], Joyce Rauch [d], Sylvie Lesage [a, b, *]

[a] Immunology-Oncology Section, Maisonneuve-Rosemont Hospital, Montréal, Québec, H1T 2M4, Canada
[b] Département de Microbiologie, Infectiologie et Immunologie, Université de Montréal, Montréal, Québec, H3C 3J7, Canada
[c] Mitacs, Computer Research Institute of Montreal, Montréal, Québec, H3N 1M3, Canada
[d] Division of Rheumatology, Department of Medicine, Research Institute of the McGill University Health Centre, Montréal, Québec, H3G 1A4, Canada
[e] Division of Immunology and Viral Infections, Institut de Recherches Cliniques de Montréal, Montréal, Québec, H2W 1R7, Canada
[f] Département de Médecine, Université de Montréal, Montréal, Québec, H3T 1J4, Canada

ARTICLE INFO

Article history:
Received 21 November 2014
Received in revised form
12 January 2015
Accepted 13 January 2015
Available online 24 January 2015

Keywords:
Autoantibodies
Affinity maturation
NOD congenic mice
Transgenic model
Autoimmune diabetes
Systemic lupus erythematosus

ABSTRACT

Autoimmune diseases result from a break in immune tolerance leading to an attack on self-antigens. Autoantibody levels serve as a predictive tool for the early diagnosis of many autoimmune diseases, including type 1 diabetes. We find that a genetic locus on mouse chromosome 12 influences the affinity maturation of antibodies as well as autoantibody production. Thus, we generated a NOD.*H2*^{k} congenic strain bearing B10 alleles at the locus comprised within the *D12Mit184* and *D12Mit12* markers, which we named NOD.*H2*^{k}-*Chr12*. We determined the biological relevance of the *Chr12* locus on the autoimmune process using an antigen-specific TCR transgenic autoimmune mouse model. Specifically, the 3A9 TCR transgene, which recognizes a peptide from hen egg lysozyme (HEL) in the context of I-A^k, and the HEL transgene, which is expressed under the rat-insulin promoter (iHEL), were bred into the NOD.*H2*^{k}-*Chr12* congenic strain. In the resulting 3A9 TCR:iHEL NOD.*H2*^{k}-*Chr12* mice, we observed a significant decrease in diabetes incidence as well as a decrease in both the quantity and affinity of HEL-specific IgG autoantibodies relative to 3A9 TCR:iHEL NOD.*H2*^{k} mice. Notably, the decrease in autoantibodies due to the *Chr12* locus was not restricted to the TCR transgenic model, as it was also observed in the non-transgenic NOD.*H2*^{k} setting. Of importance, antibody affinity maturation upon immunization and re-challenge was also impeded in NOD.*H2*^{k}-*Chr12* congenic mice relative to NOD.*H2*^{k} mice. Together, these results demonstrate that a genetic variant(s) present within the *Chr12* locus plays a global role in modulating antibody affinity maturation.

1. Introduction

Autoimmune diabetes results from the immune-mediated destruction of the insulin-secreting beta cells present within the pancreatic islets. Interestingly, molecular pathways associated with diabetes susceptibility are concordant between humans, the NOD mouse and the BB rat, which all spontaneously develop autoimmune diabetes [1–3]. In particular, the NOD mouse has been a key tool to investigate the etiology as well as the genetic susceptibility of type 1 diabetes, revealing an interesting parallel with susceptibility to type 1 diabetes in humans [1,4–8]. Consequently, validation of linkage studies for type 1 diabetes susceptibility has been performed in NOD congenic strains [6]. One notable advantage provided by the generation of the congenic lines and sublines is that it allows for significant restriction of the list of candidate genes associated with autoimmune disease susceptibility.

To explore defects in antigen-specific immune tolerance, we exploit the 3A9 TCR transgenic system, in which the TCR recognizes a peptide from hen egg lysozyme (HEL) in the context of I-A^k [9–17]. Both the HEL transgene, which is expressed under the rat-

* Corresponding author. Hôpital Maisonneuve-Rosemont, Centre de Recherche, 5415 Boul. de l'Assomption, Montréal, Québec, H1T 2M4, Canada. Tel.: +1 514 252 3400x4649; fax: +1 514 252 3569.
E-mail addresses: roxanne.collin@umontreal.ca (R. Collin), vdugas@mitacs.ca (V. Dugas), g.chabot.roy@gmail.com (G. Chabot-Roy), david.salem@mail.mcgill.ca (D. Salem), astrid.zahn@ircm.qc.ca (A. Zahn), javier.marcelo.di.noia@ircm.qc.ca (J.M. Di Noia), joyce.rauch@mcgill.ca (J. Rauch), sylvie.lesage@umontreal.ca (S. Lesage).

http://dx.doi.org/10.1016/j.jaut.2015.01.007
0896-8411/

insulin promoter (iHEL) and the 3A9 TCR transgene were initially backcrossed to two different genetic backgrounds, namely B10.BR and NOD.*H2*^{k}. Diabetes-resistant B10.BR mice are C57BL/10 mice that are congenic for the same H2^k MHC locus as NOD.*H2*^{k} mice [10,18]. Expectedly, the incidence of diabetes is relatively low in 3A9 TCR:iHEL B10.BR mice, which carry the B10.BR diabetes resistant genetic background. Although non-transgenic NOD.*H2*^{k} mice do not spontaneously develop diabetes [19], they carry all other non-MHC susceptibility loci, such that a high proportion of 3A9 TCR:iHEL NOD.*H2*^{k} mice develop diabetes [10]. Therefore, this model can be used to unravel antigen-specific modes of immune tolerance as well as to define non-MHC susceptibility loci linked to autoimmune diabetes susceptibility.

Serum insulin autoantibody levels are associated with autoimmune diabetes susceptibility and they serve as a predictive value for the onset of diabetes in both humans and mice [20–23]. Interestingly, there is growing evidence that autoantibodies are not simply bystanders in the disease process. Indeed, maternally transmitted autoantibodies contribute to diabetes onset in NOD offspring [24,25]. Similarly, maternally-derived anti-HEL antibodies contribute to the autoimmune diabetes pathology observed in the 3A9 TCR:iHEL NOD.*H2*^{k} mouse model [26]. These autoantibodies most likely arise as a consequence of a break in immune tolerance conferred by the NOD genetic background, as neither the C57BL/6 nor the 3A9 TCR:iHEL B10.BR diabetes-resistant strains present with islet-specific pathogenic autoantibodies [10,27]. Together these data suggest a role for genetically controlled autoantibody levels in the predisposition to autoimmune diabetes.

The genetic regulation of autoantibody production has been studied in the context of other diseases, namely in systemic lupus erythematosus (SLE). For instance, a genetic cross between NZW, NZB and BALB/c strains, where the NZW strain is prone to SLE, demonstrated that the *Nbwa1* quantitative trait locus (QTL) on mouse chromosome 12 is linked to high levels of anti-nuclear autoantibodies [28]. Interestingly, autoimmune diabetes and SLE share genetic susceptibility [29]. Furthermore, the NOD mouse not only develops insulin autoantibodies [20,30] but also develop antinuclear antibodies along with other tissue-specific autoantibodies [27,31]. In association with their prominent autoimmune-prone nature, NOD mice appear to exhibit a general propensity for producing autoantibodies.

In this study, we determine the effect of genetic modulation on antigen-specific autoantibody production in H2^k mice using both the 3A9 TCR:iHEL mouse model of antigen-specific autoimmune diabetes and non-TCR transgenic mice. Particularly, we develop a congenic strain to define the impact of the B10.BR locus on mouse chromosome 12 (*Chr12*), which coincides with the *Nbwa1* locus. We find that, in addition to conferring partial diabetes resistance in the 3A9 TCR:iHEL NOD.*H2*^{k} model, this locus regulates the affinity maturation of antibodies as well as autoantibody production.

2. Material and methods

2.1. Mice

3A9 TCR transgenic and iHEL transgenic mice, where HEL is expressed under the rat insulin promoter, on B10.BR and NOD.*H2*^{k} (hereafter denoted as NOD^k) backgrounds have been previously described [10]. The congenic strains were obtained by backcrossing B10.BR mice to the 3A9 TCR NOD^k parental strain for eight generations, maintaining mice heterozygous for the B10.BR allele at the *D12Mit184* marker. The iHEL transgene was introduced at the fifth backcross. At the eighth backcross, mice were maintained by brother-sister mating. Fine-mapping by PCR reaction allowed us to determine that the final congenic interval of B10 origin is located between the markers *D12Mit184* and *D12Mit12*. All NOD^k.B10-(*D12Mit184-D12Mit12*) congenic mice, hereafter named NOD^k-*Chr12*, were used after the eighth generation backcross. An illumina low-density linkage, serviced through the Center for Applied Genomics at the Hospital for Sick Children, showed the absence of B10 contamination outside the *Chr12* interval. All of the mouse strains were maintained at the Maisonneuve-Rosemont Hospital animal house facility (Montreal, Canada). The Maisonneuve-Rosemont Hospital ethics committee, overseen by the Canadian Council for Animal Protection approved the experimental procedures.

2.2. Diabetes incidence study

Female 3A9 TCR:iHEL mice were monitored daily for overt signs of diabetes (wet cage, hunched posture) and every two weeks for urine glucose levels using Diastix (Bayer, Toronto, Ontario, Canada) starting at 8–12 weeks of age. After two successive positive Diastix tests, overt diabetes was confirmed by blood glucose levels higher than 17 mmol/L. The age of diabetes onset is set at the first detection of elevated urine glucose levels. The mice were sacrificed within one week of detection of high blood glucose or when they reached more than 34 weeks of age. At culling, tail DNA was collected to verify the genotype of the mouse. The serum was collected. The pancreas was conserved in formalin for at least 48 h at room temperature before being sent for paraffin inclusion. The 3A9 TCR:iHEL NOD^k female mice included in the diabetes incidence study are littermate controls from 3A9 TCR:iHEL congenic mice that carried homozygous NOD alleles at the *Chr12* locus.

2.3. Histology

H&E staining was performed on 5–7 μm sections of pancreas cut from paraffin blocks. One histology slide per mouse, containing 1 to 3 non-successive cuts per slide were scored for infiltration as previously described [32], and according to the following scale: 0 = no infiltration, 1 = peri-insulitis, 2 = infiltration <50%, 3 = infiltration > 50%, 4 = complete infiltration.

2.4. Anti-HEL, anti-OVA and total Ig ELISA

Total IgG and total IgM antibodies were measured by ELISA according to the manufacturer's protocol (Bethyl Laboratories Inc., Montgomery, TX, USA). Serum anti-HEL or anti-OVA IgG, IgM, IgG_1 and IgG_{2c} levels were measured by ELISA on Nunc Maxisorp plates (Thermo Fisher Scientific) coated with 100 μg/ml of OVA or HEL protein prepared in $NaHCO_3$ at pH 9.5. Following incubation with serial dilutions of serum, plates were washed and incubated with goat anti-mouse IgG-HRP (Biolegend, clone poly4053), goat anti-mouse IgM-HRP (VWR), goat anti-mouse IgG_{2c}-HRP (Southern Biotech) or with rat anti-mouse IgG_1-biotin (Biolegend, clone RMG1-1) followed by avidin-HRP (Biolegend). Plates were developed with 3,3′,5,5′ − tetramethylbenzidine (TMB) substrate and read at 450 nm. A reference pool of sera from diabetic and non-diabetic TCR:iHEL mice was set to contain 1 arbitrary unit in anti-HEL antibody level, and a pool of non-transgenic mice immunized with OVA was set to contain 1 arbitrary unit in anti-OVA antibody level. Note that IgG_{2c} levels were quantified as NOD mice do not express IgG_{2a} [33].

2.5. Affinity ELISA

Sodium thiocyanate (NaSCN) displacement ELISA was performed [34]. Briefly, serum samples were diluted at similar concentrations, based on standard ELISA results. Serum dilutions were incubated overnight in a HEL- or OVA-coated plate. Plates were