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To B or not to B: Role of B cells in pathogenesis of arthritis in HLA transgenic mice

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Abstract

Population studies have shown that amongst all the genetic factors linked with autoimmune disease development, MHC class II genes are the most significant. Experimental autoimmune arthritis resembling human rheumatoid arthritis (RA) can be induced in susceptible strains of mice following immunization with type II collagen (CIA). We generated transgenic mice lacking endogenous class II molecules and expressing various HLA genes including RA-associated, HLA-DRB1*0401 and HLA-DQ8, and RA-resistant, DRB1*0402, genes. The HLA molecules in these mice are expressed on the cell surface and can positively select CD4+ T cells expressing various Vβ T cell receptors. Endogenous class II invariant chain is required for proper functioning of the class II transgene. Arthritis development in transgenic mice is CD4+ and B cells dependent. Studies in humanized mice showed that B cells are required as antigen presenting cells in addition to antibody producing cells for the development of CIA. The transgenic mice expressing *0401 and *0401/DQ8 genes developed sex-biased arthritis with predominantly females being affected, similar to that of human RA. Further, the transgenic mice produced autoantibodies like rheumatoid factor and anti-cyclic antibodies. Antigen presentation by B cells leads to a sex specific immune response in DRB1*0401 mice suggesting a role of B cells and HLA-DR in rendering susceptibility to develop arthritis in females.

Keywords

MHC polymorphism; HLA transgenic mice; Rheumatoid arthritis; B cells; antigen presentation

Rheumatoid arthritis (RA) is an autoimmune disease characterized by inflammation of the synovial lining of joints. Familial clustering of rheumatoid arthritis and other autoimmune diseases and their occurrence in monozygotic twins suggest that genetics plays an important role in susceptibility to autoimmunity [1–3]. Predisposition to rheumatoid arthritis has been linked to the major histocompatibility complex (MHC) class II HLA-DRB1 locus [4–6]. Among the HLA-DR4 genes, DRB1*0401 (Dw4), DRB1*0404 (Dw14), and DRB1*0405 (Dw15) alleles confer predisposition to develop RA while DRB1*0402 (Dw10) does not [4, 5]. This association has been explained on the basis of differences in the third hypervariable region (HV3) of the DRB1 alleles and is called the ‘shared epitope hypothesis’ [5, 7]. Thus

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DRB1 alleles sharing the amino acid motif Leu/Gly/Arg/Lys/Ala (L/Q/R or K/A) at position 67, 70, 71, and 74 of the HV3 region of DRB1*0401 render susceptibility to develop RA, while the sequence motif of I/D/E/A expressed at positions 67, 70, 71, and 74 (as expressed in DRB1*0402) confers resistance to RA. HLA-DQ occurs in linkage disequilibrium with DR genes and thus is inherited enbloc as a haplotype [8]. The DQB1*0301 (DQ7) and DQB1*0302 (DQ8) genes are in linkage disequilibrium with DR4 alleles. RA patients in India were found to be predominantly of the DQ8/DR4 haplotype [9] while studies in Caucasian population showed an association of severity of arthritis with DQ7/DR4 [10]. These data, although controversial, support a role for HLA-DQ alleles in genetic predisposition to RA. Recently, genome wide association studies have shown that among all the factors associated with RA, MHC shows the strongest and most important association compared to other genetic factors. The majority of single nucleotide polymorphisms (SNP) associated with rheumatoid arthritis were located in the HLA region, suggesting that HLA has the greatest influence on RA phenotype [11, 12]. Despite a number of studies demonstrating association of class II molecules with rheumatoid arthritis and other autoimmune diseases, the mechanisms to explain these associations remain obscure.

Since autoimmune diseases are generally heterogeneous, different mechanisms that implicate HLA molecule itself by virtue of its role in the generation of immune response or as secondary molecule have been hypothesized to explain HLA gene association with diseases; [13]. Other mechanisms by which HLA molecules could facilitate the development of some diseases is by influencing the T cell repertoire [14] or forming the basis for selection of T cell repertoire in the thymus [15]. However, studies to resolve this in humans have been hampered by the following 1) lack of knowledge of the autoantigens or very low frequency of autoreactive cells, 2) huge genetic variation between individuals, 3) the linkage disequilibrium of HLA class II alleles, DR and DQ, makes it difficult to interpret the association with a haplotype or specific allele and 4) by the time most patients are diagnosed, initial immune response to the autoantigen(s) may have subsided or expanded to other antigens.

Collagen-induced arthritis as a model for RA

Type II collagen constitutes 80–90% of the total collagen content of the hyaline cartilage found in joints, and is a genetically conserved sequestered protein and thus could be an autoantigen when presented in an appropriate immunogenetic context. An injury could potentially result in denaturing of type II collagen and the exposure of potential cryptic determinants which could initiate epitope spreading and activation of autoreactive T cells, ensuing in a severe disease. Patients with RA have been shown to produce anti-collagen type II (CII) antibodies, T cell reactivity to CII, and accumulation of CII-reactive T cells in synovial fluid, suggesting that autoreactivity to collagen might be important in pathogenesis [16–19]. Inflammatory arthritis that shares a number of clinical, serological, and radiographic features with RA in humans can be induced in mice following immunization with heterologous type II collagen [20]. Collagen induced arthritis (CIA) represents an experimental autoimmune disease dependent upon the immune response to a tissue restricted, sequestered protein where the immune and autoimmune responses are under immunogenetic regulation. Arthritis in humans and mice occurs in both large and small joints and involves both front and hind limb joints, leading to progressive destruction. Joints of arthritic animals show neoangiogenesis and pannus formation that leads to damage of the cartilage and subchondral bone. X-rays taken of joints of mice with this arthritis show the typical erosive damage as seen in the human rheumatoid arthritis. Although experimentally induced, polyarthritis in mice is symmetrical with development of anti-collagen antibodies similar to that observed in RA. Even though CIA has been used as a model for rheumatoid

arthritis for decades, there are some features of rheumatoid arthritis that are not observed in mouse model of arthritis.

Mouse models of RA have often been critiqued as having inherent immunological shortcomings. One consistent shortcoming of mouse models of RA is the fact that human but not mouse T cells express class II molecules. Since CD4 cells are activated in RA, class II positive CD4 cells can present antigen locally in a joint, exacerbating the inflammatory response, which can not occur in mouse models. Even though the histological and clinical phenotype of CIA and RA show similarities, mouse models of RA consistently fell short in generating endocrinological conditions approximating the human female biased susceptibility to RA. In addition, autoantibodies like rheumatoid factor (RF) and anti-citrullinated peptide antibodies (ACPA) that are diagnostic markers for RA are not produced by mouse models of arthritis. These fundamental immunological differences have frequently left mouse CIA models suspect in the eyes of many clinicians. The advent of mouse class II knock-out mice expressing human HLA-DR and HLA-DQ transgenes has significantly advanced the understanding of the role of individual HLA class II molecules in various clinical conditions including RA. Recent CIA models generated using HLA transgenic mice have overcome most of the known deficiencies of the arthritis mouse models with endogenous class II molecules.

HLA transgenic mice as model for rheumatoid arthritis

The first model of autoimmunity to determine the role of class II molecules was established by using transgenic mice carrying genes from RA susceptible haplotype DQA1*0301, DQB1*0302 (DQ8) [21]. Various studies have shown that invariant chain (Ii) is essential for proper assembly and transport of MHC class II molecules to the cell surface [22, 23]. To determine if the expression of HLA-DQ8 was dependent on the accessory role of Ii, we generated mice lacking endogenous class II molecules (Aβo) and expressing HLA-DQ8 (Aβo.DQ8) that are Ii sufficient and those expressing DQ8 with disrupted Ii gene, Aβo.DQ8.Ii−/− mice. Analysis of expression of DQ8 molecules on the cell surface by FACS showed a much reduced expression with fewer percent of cells expressing DQ8 in DQ8.Ii−/− mice compared to DQ8 mice. Since Aβo mice harbor less than 1% of CD4+ cells, we evaluated if expression of DQ8 led to positive selection of CD4+ cells. Introduction of DQ8 transgene in Aβo mice rescued CD4 cells in DQ8 mice but not in DQ8.Ii−/− mice. Both strains positively selected CD8 and B220+ cells although DQ8.Ii−/− mice exhibited much lower numbers of B cells compared to DQ8 mice (Figure 1A). Since a reduced number of mature CD4 T cells were observed in DQ8.Ii−/− mice, we determined Vβ T cell repertoire in both transgenics. DQ8.Ii−/− mice showed a reduced number of certain the Vβ T cells compared to DQ8 mice. These studies confirmed previous findings on the role of Ii chain and suggested that Ii chain is important for expression of HLA transgene in mice, thereby affecting cell selection.

Transgenic HLA-DQ8 and DQ8Ii−/− mice were tested in our collagen-induced arthritis protocol. Upon immunization with bovine type II collagen, DQ8 transgenic mice generated autoantibodies to mouse type II collagen, and approximately 60–75 % of them developed severe inflammation and swelling which progressed to severe arthritis leading to joint deformity [21, 24]. Histological examination of the arthritic hind limbs showed cellular infiltration, marked synovitis consisting of synovial cell hyperplasia, erosion of articular cartilage and subchondral bone. Thus, disease in these DQ mice was similar to the human linkage studies in RA. On the other hand, DQ8 Ii−/− mice were resistant to develop arthritis, with only 20% mice developing a milder arthritis with delayed onset as compared to DQ8 mice (Figure 1B). None of the DQ8.Ii−/− mice developed any measurable amounts of anti-CII antibodies. Cellular response to CII and known DQ8-restricted CII-derived

peptides [25] was also significantly reduced in DQ8.Ii−/− mice when compared to DQ8 mice, as observed in vitro in a recall response to the antigens (Figure 1). These observations suggested a crucial role of Ii chain in cell selection and the proper functioning of HLA molecules in transgenic mice. In addition to the chaperone molecule such as Ii, costimulatory molecules like CD28 also function normally in transgenic mice as observed by studies using DQ8.CD28−/− mice [26]. These studies show that the HLA transgenes work with endogenous molecules suggesting the transgenic mice are functional and can provide good models to study various HLA-associated human diseases.

Further, we have generated double transgenic mice expressing HLA-DR and -DQ genes to simulate human haplotype. Using DR/DQ transgenic mice, we showed that DRB1 polymorphism modulates DQ-restricted CIA [27]. Expression of RA-non associated genes, DR2 and DRB1*0402, protect mice from DQ8-restricted arthritis while *0401 is permissive for DQ8-restricted arthritis and enhances disease incidence [28, 29]. Thus these transgenic mice could provide a reliable model to study the pathogenesis of RA and to determine the role of HLA genes individually in pathogenesis of RA.

Collagen-induced arthritis in HLA transgenic mice is CD4 and B cell dependent

Most of the published studies in RA and CIA have suggested an involvement of T cells, especially $CD4^+$ T cells, in pathogenesis. These include 1) infiltration of the inflamed synovium with predominantly T cells, 2) improvement in joint disease manifestations of arthritis following treatment with depleting CD4 antibody, and 3) multiple oligoclonally expanded CD4 T cells within the rheumatoid joint [30–32]. Presence of anti-type II collagen reactive CD19+ B cells and oligoclonal expansion of T cells in RA joints is thought to be driven in part by type II collagen (CII) [33, 34]. In CIA, CII-specific $CD4^+$ T cells have been reported to be fundamental in initiation and perpetuation of the disease [35]. Using DQ8 transgenic mice lacking CD4 or CD8 molecules, we showed that CD4 cells are essential for the initiation of CIA while CD8 cells may be involved in regulation of disease [36].

To determine the requirement of B cells in the pathogenesis of arthritis, DQ8 mice deficient in B cells, DQ8.umt, were generated and tested in our CIA protocol. DQ8.umt mice are resistant to arthritis [37]. Since B cells are required for antibody production, DQ8.umt mice were immunized with CII and sera from arthritic positive mice was injected. Only a few DQ8.umt mice developed transient arthritis suggesting B cells have an important role in the pathogenesis of arthritis that extends beyond antibody production.

B cells in CIA susceptible versus resistant transgenic mice

We have used double transgenic mice expressing DRB1*0401/H2Aq and DRB1*0402/H2Aq genes to determine if DR4 polymorphism can influence humoral response and CIA susceptibility [28, 38]. Observations in these transgenic mice suggest that DRB1 polymorphism influences H2Aq-restricted CIA similar to that observed with DR/DQ mice. To determine if B cell reactivity is different in CIA susceptible DRB1*0401 and resistant DRB1*0402 mice, we tested antibodies to cyanogen bromide (CB) fragments of type II collagen in *0401/H2q and *0402/H2q transgenic mice. Both transgenic strains responded to the same T cell immunodominant peptide of CII, 254–273, but B cell reactivity showed differential response [38], Figure 2A). Arthritis-susceptible mice produced higher levels of autoantibodies compared to resistant mice. Further, we measured antibody reactivity against cyanogen-bromide digested CII peptides in sera of transgenic mice primed with CII. No significant difference was observed in the pattern of antibody binding measured against CB

fragments of CII between the two transgenic strains, suggesting that the type of anti-collagen antibody response was primarily determined by H2-A (homologue of HLA-DQ) molecule.

B cell epitopes can differ from T cell epitopes in CIA susceptible mice

T cell epitopes of human CII have been characterized in HLA-DQ8 transgenic mice [25]. We used some of the immunodominant T cell peptides known to be immunogenic in different strains of mice to test the reactivity of B cells in DQ8 mice. Two of the immunodominant DQ8-restricted T cell epitopes (peptides CII-7 (184–203) and CII-17 (284–303) showed poor B cell reactivity (Figure 2B). Collagen-induced arthritis susceptible mice are good responders of CII-17 while resistant mice are not [25]. These data suggest that a strong T cell epitope may not necessarily be pathogenic, as it may not generate autoantibodies. The most immunodominant T cell peptide, CII-44 (564–573), showed very mild reactivity with B cells while the peptide with poorest T cell stimulation showed high reactivity. One of the peptides, HII-43 (544–563), was a strong T and B cell epitope. These data suggest that 1) T and B cell epitope might differ and 2) B cell epitope spreading may be one of the factors involved in CIA in transgenic mice.

HLA transgenic mice develop sex-biased arthritis

Recently, we have generated mice lacking complete endogenous MHC class II molecules (AEo) and expressing HLA-DRB1*0401 and DQ8 [29]. In these mice a subset (5–15%) of CD4 T cells express class II molecules similar to that known in humans, although this feature is not observed in mice. CD4 T cells expressing human class II transgenes can present peptides to other CD4 cells suggesting a role of activated CD4 cells infiltrating joints in the pathogenesis of arthritis. Immunization of DRB1*0401 mice led to the development of arthritis predominantly in females with a female to male ratio of 3:1 [29]. To simulate human haplotype, double transgenic mice expressing RA-associated DQ8 and *0401 alleles were the AE−/− background. Transgenic mice expressing *0401/DQ8 develop arthritis predominantly in females, with a female to male ratio of 2:1, similar to the sex-bias known in human RA [28]. On the other hand, DQ8 mice developed arthritis with similar incidence in male and female mice (Figure 3A). This led us to hypothesize that DR4 renders sex-bias in development of arthritis. Female mice generated a higher cellular response to CII in vivo and in vitro in *0401 and *0401/DQ8 strains confirming a role of DR4 in sex-bias of arthritis (Figure 3B, [29]). Observations in these transgenic mouse models show that mimic human disease; both require presentation of an arthritogenic epitope(s) by HLA class II molecules to CD4 T cells, leading to proliferation of autoreactive cells and production of autoantibodies by B cells, subsequently leading to joint pathology. A role of hormones is suggested in the pathogenesis of RA and CIA in AEo DR4/DQ8 transgenic mice

The observations in transgenic mice and human data led us to hypothesize that DQ polymorphism may render susceptibility to develop arthritis while DRB1 polymorphism may be involved in modulation of disease. From the studies on DQ and DR transgenic mice, it can be extrapolated that gene complementation or interaction between DQ and DR molecules mediates susceptibility to RA in the human. Depending on the haplotypes carried by an individual, they could be susceptible to severe or mild disease. A homozygous haplotype for predisposing DQ and permissive DR will lead to severe disease. Also, heterozygous RA-susceptible haplotypes will result in very severe disease since there will be two predisposing DQ molecules. However, one predisposing and one protective haplotype should show less severity and low incidence.

HLA transgenic arthritic mice produce RF and ACPA antibodies

Antibodies produced by B cells like RF (an auto-antibody directed at the Fc part of IgG) are a hallmark of patients with rheumatoid arthritis. Autoantibodies have been shown to be important for pathogenesis of arthritis in RA; about 80% of patients are RF+. Although the classical auto-antibody associated with RA is RF, the sensitivity and specificity of RF for RA are low [39]. A break-through came with the discovery of auto-antibodies that have a similar sensitivity as RF but a much higher specificity, anti-cyclic citrullinated peptide antibodies (ACPAs). Various studies have shown that both RF and ACPAs precede the onset of the arthritis. In RA patients, B cell depletion therapy has been shown to ameliorate the clinical manifestations of rheumatoid arthritis [40]. A critical role of B cells has been demonstrated in several models of autoimmune diseases that include lupus, arthritis and diabetes [41–43].

In experimental model of arthritis, transfer of autoantibodies can induce transient and mild arthritis [44]. Most of the mouse models of arthritis do not produce rheumatoid factor and ACPAs. Using DQ8 mice deficient in CD8, we showed for the first time that arthritic transgenic mice produce autoantibodies like rheumatoid factor and anti-nuclear antibodies [36]. These studies also suggested that CD8 cells could be regulatory cells that may act via modulation of B cell activity. Our recent studies with CIA in AEo.DRB1*0401 and DQ8 mice showed that arthritic mice produce RF, IgG and IgM, and ACPAs as well as antibodies to self CII (Figure 3C,3D) [28, 29]). The levels of the auto-antibodies correlate with the severity of disease. Further, female mice produced much higher levels of auto-antibodies compared to males, which could be due to higher numbers and proliferation of B cells in vivo in CII-immunized females compared to male mice, (Figure 3E). These data suggest that B cells are hyperactive in females and may contribute to immune response leading to sex-bias in disease development. We have utilized these humanized mice to further investigate the role of B cells in context of RA associated HLA genes.

B cells are important for antigen presentation in arthritis

B cells can function in many ways; they can act as antigen-presenting cells and activate T cells, secrete pro-inflammatory cytokines, produce antibodies. While the role of B cells in antibody production is well established, their role in presenting antigens to naïve CD4+ T cells is somewhat controversial [45, 46]. Mouse studies have suggested an important role for B cells as antigen presenting cells to autoreactive T cells [47]. We used DQ8.umt mice to study if B cells are required as antigen presenting cells for development of CIA. DQ8.umt mice are resistant to developing arthritis which can be explained partly by the absence of autoantibodies. However, DQ8.umt mice generated a significantly lower cellular response to CII and CII-derived peptides compared to DQ8 mice despite an increased number of Mac-1 positive cells when compared to DQ8 mice, suggesting that B cells may be critical as antigen presenting cells (APCs) for CIA. Using antigen-specific DQ8-restricted T cell hybridomas, we compared antigen presentation efficiency of B cells and dendritic cells. The data showed that B cells can present CII-peptide although DCs are much more efficient APCs [37]. To determine efficiency of B cells in presenting antigenic peptides to CD4 T cells, we used known DR4 and DQ8 restricted CII-peptides and *0401 and *0401/DQ8 transgenic mice. B cells isolated from primed *0401 and *0401/DQ8 mice can present DR4-restricted, CII-14 (254–273), as well as DQ8-restricted, CII-17 (284–303), peptide. In vitro presentation of DR4-restricted CII peptide by B cells generated more robust response than presentation of DQ-restricted 284–303 peptide (Figure 3F). In vitro *0401/DQ8 mice produce higher amounts of TH17 and modulating cytokines like IL-13 and IL-10 in response to DR4-restricted CII 254–273 compared to DQ8-restricted CII 284–303 (Figure 3G). These studies reinforced the role of B cells in antigen presentation in pathogenesis of arthritis.

Since B cell epitopes may be different than T cells, it could lead to epitope spreading, generating a strong cellular and humoral response to the arthritogenic antigen(s).

DRB1*0401 and DRB1*0401/DQ8 mice produce high levels of Th17 and B cells modulating cytokines

DRB1*0401/DQ8 and DRB1*0401 mice are susceptible to CIA while DRB1*0402 and DRB1*0402/DQ8 mice are not. We tested if CIA susceptible mice generate a different CII-specific immune response compared to resistant strains. Our data showed that CIA susceptible mice produced significantly higher levels of proinflammatory cytokines, IL-17, IFN-γ, IL-1, TNF-α and IL-23 compared to CIA resistant mice when challenged with CII [28, 48]. Transgenic *0401 and *0401/DQ8 mice also produced high levels of immunomodulatory cytokines, IL-3 and IL-13. Our findings are supported by higher levels of IL-13 in RA patients than healthy individuals and its correlation with CCP positivity [49]. IL-3, found significantly increased in *0401.DQ8 mice compared to *0402/DQ8 mice, can regulate proliferation and survival of neutrophils resulting in production of chemokines ensuing a pro-inflammatory response [50]. These neutrophils can release B cell activating factor (BAFF), which is important for proliferation and maturation of B cells. This could explain increased amounts of autoantibodies observed in *0401 and *0401.DQ8 mice compared to CIA resistant *0402 and *0402.DQ8 mice [28].

DRB1*0401 and *0401/DQ8 mice immunized with CII show sex-bias proliferation of B cells and develop sex-biased arthritis. We tested if the immune response generated by B cells following CII or CII-peptide immunization is different between sexes. Arthritis susceptible mice produced cytokines that modulate B cell function, so we determined cytokine profile following presentation of DR and DQ restricted peptides by B cells in *0401/DQ8 mice. Interestingly, when DR4 restricted peptide is presented by B cells, there is a high production of Th1, Th17, immunomodulatory cytokines like IFNg, IL-17, IL-3, IL-13 and IL-10 and chemokines in vitro (Figure 3G [51]). IL-13 increases the expression of MHC antigens and stimulates proliferation of, and antibody production and Ig class switching by B cells, while IL-10 decreases expression of these antigens on activated monocytes [52–54]. Comparatively, when DQ-restricted peptide 284–303 is presented by B cells, amounts of cytokines produced are 10–25 fold less with IFNγ being the highest followed by IL-17. These observations suggest that presentation of DR-restricted peptides by B cells produce a cytokine milieu that supports inflammation by increasing cytokine and chemokine production which can attract other inflammatory response cells. DQ-restricted peptide presentation increases the Th1 and Th17 response that adds to the inflammatory loop.

Differential role of B cells in both sexes

To understand differences in the basic immune response that can lead to a sex-biased susceptibility to arthritis development in transgenic mice, we tested the DRB1*0401 and DQ8 transgenic mice for overall cellularity and also cellular composition in spleen. DRB1*0401 female mice had higher absolute number of total spleen cells than male mice (Figure 4A). The most significant difference seemed to be in the presence of CD4 and B cells, which were higher in number in female mice of the DR4 strain. This data reproduces human findings, where higher absolute numbers of CD4 cells in women compared to men have been described to be under genetic control [55]. However, these differences were not observed in DQ8 mice. From this data one can speculate a role of DR gene and probably other factors in sex-specific cellularity. DRB1*0401 mice were used to determine if DR4-restricted antigen presentation and autoantibody production by B cells results in sex-bias in arthritis in these mice. Sorted CD4+ and B cells from CII-derived peptide 254–273 primed *0401 mice were cultured in vitro. As shown in Figure 4B, B cells from female mice

presented antigen 3 times more efficiently than male mice. Using *0401/DQ8 transgenic mice, the presentation of the immunodominant DR-restricted and DQ-restricted peptide by B cells was compared between sexes. In double transgenic mice, *0401/DQ8, there was no difference between male and female mice in the DR4-restricted antigen presentation by B cells, however males mounted a significantly stronger response to DQ8-restricted peptide CII-284–303 compared to females (Figure 4B). Since DR4-restricted CII peptide generated a more robust response in females when presented by B cells, we compared the expression of DR4 in both sexes. Females had a trend towards higher expression of DR4 in B cells compared to males. To understand if hormones have a role in expression of HLA-DR, male mice were implanted with exogenous estradiol pellets and analyzed for expression of DR transgene by FACS. Male mice receiving exogenous estradiol showed a much higher expression of DR4 [51] and generated a more robust response to DR4-restricted CII 254–273 compared to male mice without exogenous estradiol (Figure 4C).

The above observations clearly showed that antigen presentation by B cells may be important in generating inflammatory response in CIA susceptible transgenic mice. We further determined if DR4-restricted antigen presentation by B cells leads to a different cytokine profile in both sexes. Comparison between sexes showed that males produced higher amounts of IL-17 and IL-10 while females generated significantly higher amounts of IL-13 when B cells presented CII 254–273 in a recall response (Figure 4D). These observations suggest that females generate Th17 and B cell modulating response while males generate Th17 and a regulatory cytokine like IL-10. Regulatory B cells are known to produce IL-10 [48]. Transgenic *0401 males showed higher levels of regulatory B cells compared to females [51]. Although these observations do not prove directly that B cells are involved in sex-bias, one can speculate that DR4 expression and DR4-restricted antigen presentation by B cells may have a role in enhancing immune response to arthritogenic peptides in females thus contributing to pathogenesis and sex-bias of arthritis.

B cell directed Immunotherapy in RA

All of the functions of B cells in the genesis and perpetuation of autoimmunity serves as a basis for the efficacy of B cell directed therapies. In particular, an anti-CD20 therapeutic antibody has been demonstrated to be highly effective in the treatment of B cell malignancies as well as in various autoimmune syndromes including Rheumatoid arthritis [56, 57]. Anti-CD20 treatment eliminates a number of B cell parameters, including their APC functions, B cell cytokines, sources of autoantibody production and immune complex formation, and the direct infiltration of organs by B cells. It is not clear which of these functions may be most critical in the treatment of disease. Studies suggest that B cells are not critical in the maintenance of T cell anergy or tolerance. It would be predicted that autoimmune syndromes would arise more frequently in individuals that lack “tolerizing” B cells due to treatment. This phenomenon is not observed in treated individuals. Since CD20 is expressed on B cells from pre-B stage in the bone marrow to the mature B cell stage, these lymphocyte subsets are eliminated by anti-CD20 treatment as demonstrated by several clinical trails involving autoimmune diseases. However, antibody-secreting plasma cells are refractory to the drug since CD20 expression is down regulated in them. Depletion of CD20 cells in RA patients showed a 50% improvement in ACR response criteria supporting an important role of B cells in pathogenesis of RA. The Phase III Randomized Evaluation of Long-Term Efficacy of Rituximab in RA (REFLEX) trial demonstrated efficacy of RTX in patients with a history of non response to tumor necrosis factor inhibitors [40]. However, the mechanism by which anti-B cell treatment works is still unknown.

We have demonstrated a mechanism by which B cells in conjunction with HLA-DR4 may lead to sex-bias in arthritis. However, the data does not prove that this may be the only

phenomenon involved in sex-bias. Our studies are supported by reports of modulation of B cells by estrogen [58]. There is currently great interest in new ways to individualize therapy for patients with RA or other inflammatory rheumatic diseases [59]. Despite the known sex-bias of RA, it is unknown if the optimal therapeutic approach differs among female and male patients. Current guidelines make no distinctions based on gender in the recommendations for the use of biologic or non-biologic disease modifying therapies. Our data sheds light on how B cells behave in sex dependent manner and persuade us to look into the mechanistic differences in B cell- and dendritic cell (DC)-mediated antigen presentation and HLA-DR4-restricted immune responses that may underlie the sex-based differences in disease phenotype. If the data in DR4 humanized mice is true, then targeting therapies to these distinct cellular mechanisms differently in women versus men might improve treatment outcomes.

Concluding Remarks

Rheumatoid arthritis occurs two to three times more often in women than in men with about 70% of patients being women. Despite extensive research, the mechanisms underlying the effects of sex on the phenotype of RA are unknown. A difference in MHC-restricted presentation of synovial antigens may explain the difference in genetic load requirement and sex-bias of disease. While autoantibodies like RF and ACPAs are important diagnostic markers for RA, antigen presenting ability of B cells in sex-specific manner has not been explored. We propose that B cells are important as antigen presenting cells and may modulate immune response in the context of specific HLA molecules that are sex-specific and pathogenic. While B cells can get activated via TLRs, their antigen-presenting function is important for inflammatory response leading to onset of disease (Figure 5). An injury or constant infections can release self-antigens which when presented by a RA susceptible haplotype lead to production of proinflammatory immune response, activation of NfkB pathway, increase survival of cells and pathogenesis.

Defining the mechanism of pathogenesis in experimental models will help define sex-bias of disease. Ours and other findings bring forth the following important observations 1) sex hormones likely contribute towards pathogenic response in arthritis, 2) HLA haplotype (DR and DQ) provides the most significant association for disease phenotype, DRB1 polymorphism modulates immune response, and 3) antigen presenting cells in context of HLA alleles may define the final immune response. Humanized mice expressing HLA genes provide one way to study the role of individual genes for investigating genetic, environmental and pathogenic aspects of an autoimmune disease in a biologically relevant situation in order to address questions about optimizing treatment based on patient’s sex.

We contribute this paper to the special issue dedicated to the enormous contributions of Dr. Chella David, a pioneer in discovery of mouse major histocompatibility complex and its role in autoimmunity. Doctor David laid some of the major fundamental groundwork in discovery of mouse major histocompatibility complex [60–62]. Mice generated by Dr. David were used to define MHC-restriction that won the scientists Nobel prize [63]. He has pioneered the generation of functional mouse models of autoimmune diseases using HLA transgenic mice [21, 64–66]. Although his major focus has been autoimmune inflammatory polyarthritis, he has contributed to the understanding of immunogenetics of other autoimmune diseases like multiple sclerosis, diabetes and celiac disease [64, 66]. We are pleased to contribute to this issue for many reasons and note that this is one of a series of special issues of the Journal of Autoimmunity/Autoimmunity Reviews which recognizes distinguished figures in autoimmunology and critical subjects for review that have impact for patients [67–84]. The use of humanized mice is recognized as a major resource to define

the mechanism of pathogenesis of various autoimmune diseases. We acknowledge Dr. David's long term commitment to understanding of autoimmunity.

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A

	DQ8	DQ8.Ii-/-
IVD12	50±9	25.4±5.8
CD4+	6.7± .0.4	0.9±0.4
CD8+	15.8±1.1	19.9 ±4
B220+	41.1 ± 16.1	16±6.6

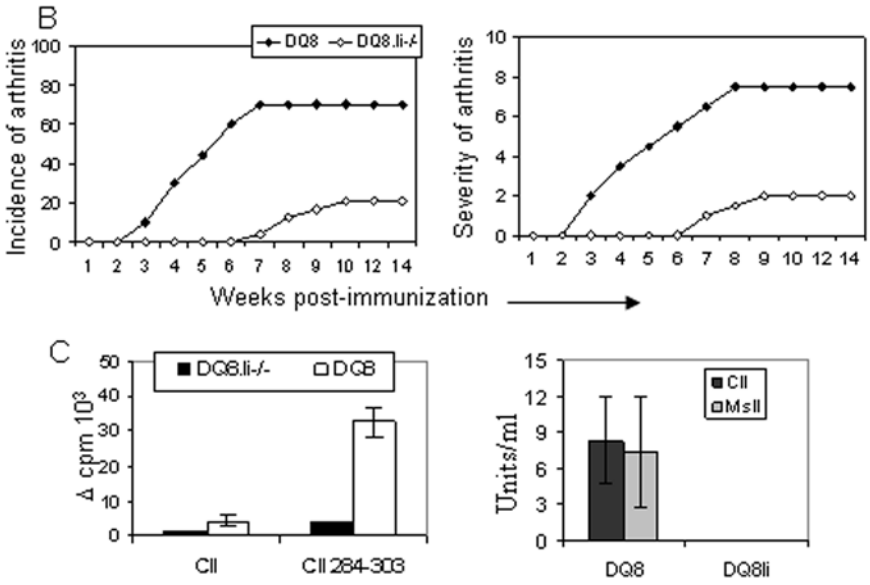

Fig 1.

Invariant chain is required for expression and functioning of HLA molecules. A) Expression of HLA-DQ8 was reduced in DQ8.Ii−/− mice compared to DQ8 mice. HLA-DQ expression was analyzed by flow cytometry after staining with conjugated antibodies and is shown as percent positive cells. Reduced expression of DQ8 leads to lower numbers of CD8 and B220+ cells in DQ8Ii−/− mice compared to DQ8 mice, while single positive CD4 cells are not rescued in absence of invariant chain. B) Incidence and severity of collagen-induced arthritis in DQ8Ii−/− and DQ8 mice. C) Cellular and humoral response in DQ8Ii−/− mice shows a significant decrease in T cell proliferation to recall response to type II collagen (CII) and antibodies to CII compared to DQ8 mice.

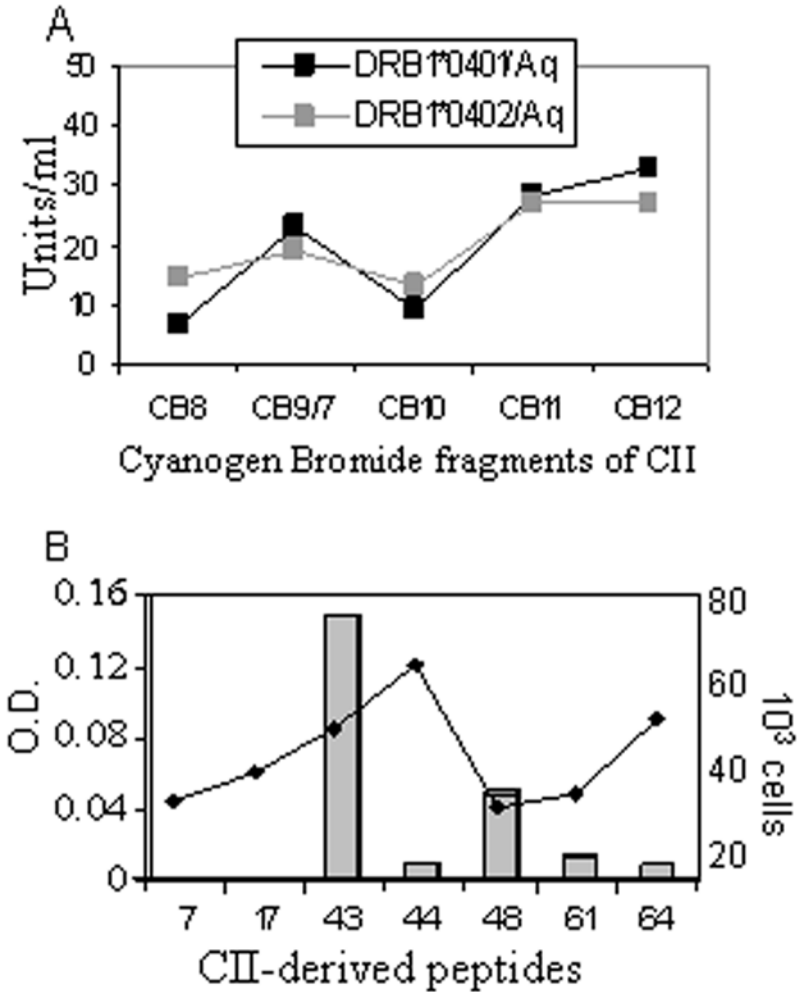

Fig 2.

B cell epitopes differ from T cell epitopes. A) B cell reactivity to cyanogen bromide fractions of CII in CIA-susceptible *0401/Aq mice and CIA-resistant *0402/Aq mice shows no significant difference. B) T cell epitopes (line) and B cells epitopes (bars) tested in DQ8 mice using type II collagen (CII) derived peptides. CII-Peptides 7=184–303, 17=284–303, 43=544–563, 44=554–573, 48=594–603, 61=714–723, 64=744–853.

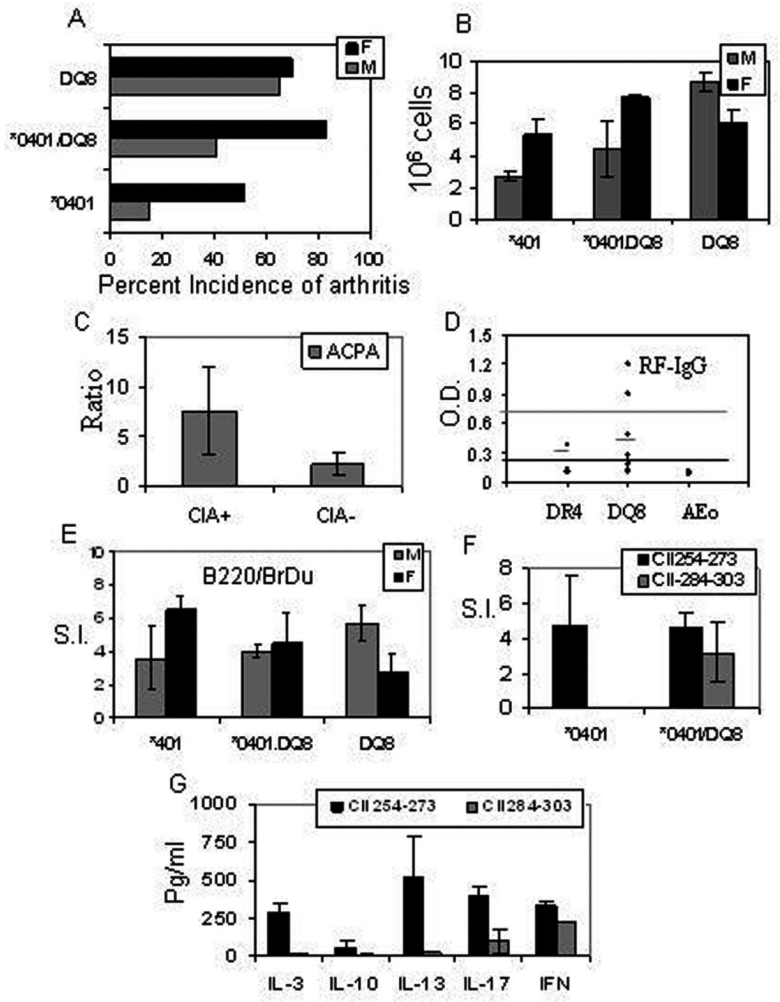

Fig 3.

HLA transgenic mice develop sex-bias arthritis and immune response. A) Incidence of collagen-induced arthritis (CIA) in male and female transgenic mice. B) In vivo proliferation of splenic cells in response to type II collagen (CII) measured by BrDu staining. Mice were given BrDu and 10 days post-immunization, numbers of various cells positive for BrDu were counted. C) Anti-cyclic peptide antibodies (ACPA) in collagen-induced arthritis (CIA) positive and negative transgenic mice. Anti-CCP antibodies are denoted as ratio of absorbance units of primed transgenic and control mice. D) IgG-rheumatoid factor titers in sera of CII-immunized transgenic mice measured by ELISA. Upper and lower lines denote positive, MRL-lpr mice, and negative, B10, controls respectively. E) In vivo proliferation of B220 cells in male and female transgenic mice. Proliferation was measured by BrDu staining. F) CD4+ cells generate higher response to *0401-restricted CII peptide 254–273 compared to DQ-restricted CII peptide 284–303 when presented by B cells isolated from primed DRB1*0401 and *0401/DQ8 mice. G) Cytokines measured from culture supernatant of *0401/DQ8 mice described in Fig 3F.

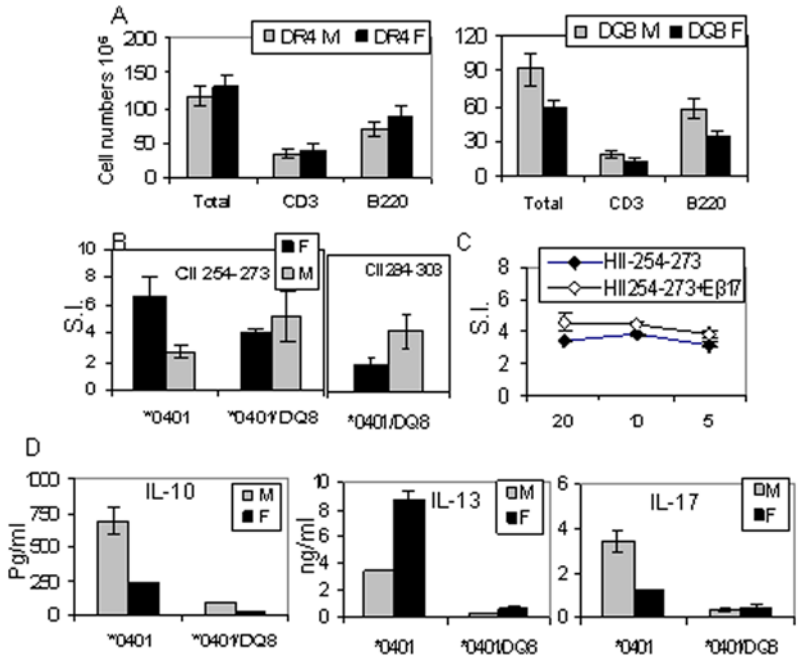

Fig 4.

Transgenic mice generate sex-biased response to CII-peptides. A) Total number of various cells in male and female *0401 and DQ8 transgenic mice. B) Female mice respond to DR4-restricted CII peptide (254–273) while males generate higher response to DQ8-restricted CII peptide (284–303). C) In vitro T cell proliferation to CII 254–273 in *0401 male mice with or without exogenous estradiol (Eβ17). D) Cytokines produced by male and female transgenic mice in response to CII peptide 254–273 when presented by B cells isolated from primed mice.

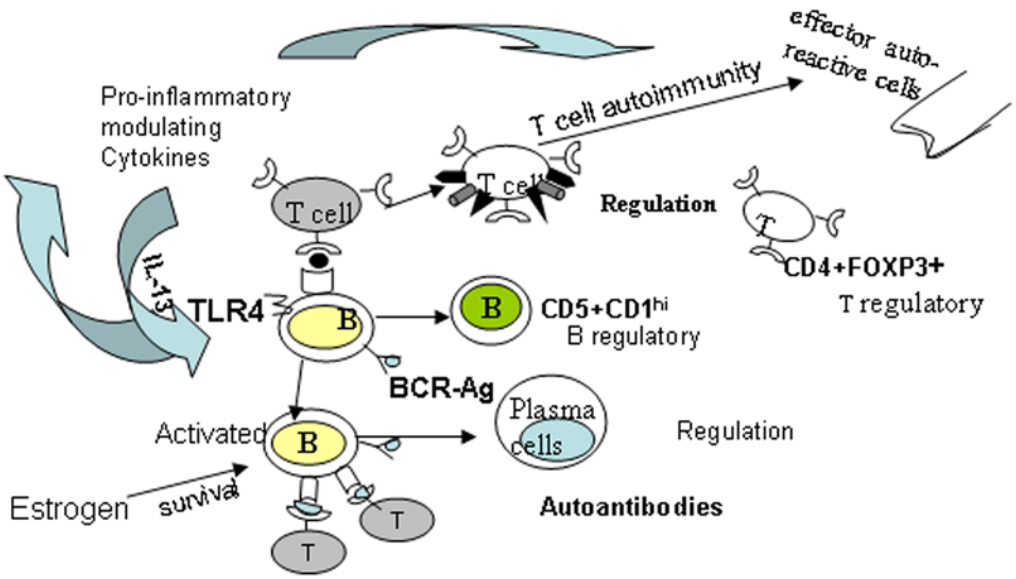

Fig 5.

A schematic diagram of the role of B cells in collagen-induced arthritis. In arthritis-susceptible mice, B cells upon antigen encounter proliferate and differentiate into plasma cells producing antibodies. B cells can get activated via adjuvant using TLR4 signaling that activates NFkB pathway leading to production of proinflammatory cytokines and chemokines by activated T cells and B cells. Since type II collagen is a self molecule, autoreactive T cells become activated leading to T cell autoimmunity. Production of B cell modulating cytokines via DR4-restricted antigen presentation can increase B cell proliferation and survival thus modulating their function. B cells can also take up antigen (self/mimic) via BCR and then process and present various processed epitopes to different T cells via MHC class II thus amplifying the T cell response. In turn these activated T cells can provide B cell help and promote B cell epitope spreading. In collagen-induced arthritis in DR/DQ transgenic mice, differential antigen presentation by DR and DQ molecules, activation of B cells, various cytokines produced by B cells upon immunization with type II collagen along with differences in regulatory B and T cells determine the outcome of disease and sex-bias in arthritis.