CD4 and CD8 T Cells in Susceptibility/Protection to Collagen-Induced Arthritis in HLA-DQ8-Transgenic Mice: Implications for Rheumatoid Arthritis

Veena Taneja, Neelam Taneja, Tawatchai Paisansinsup, Marshall Behrens, Marie Griffiths, Harvinder Luthra and Chella S. David

*J Immunol* 2002; 168:5867-5875; doi: 10.4049/jimmunol.168.11.5867
http://www.jimmunol.org/content/168/11/5867
CD4 and CD8 T Cells in Susceptibility/Protection to Collagen-Induced Arthritis in HLA-DQ8-Transgenic Mice: Implications for Rheumatoid Arthritis

Veena Taneja, Neelam Taneja, Tawatchai Paisansinsup, Marshall Behrens, Marie Griffiths, Harvinder Luthra, and Chella S. David

To investigate the role of CD4 and CD8 T cells in arthritis, we generated transgenic mice deficient in CD4 and CD8 molecules expressing RA-susceptible gene HLA-DQ8. DQ8−/− mice were resistant to developing collagen-induced arthritis (CIA). However, DQ8−/− mice developed CIA with increased incidence and more severity than DQ8 mice. Both DQ8−/− and DQ8 mice produced rheumatoid factor. In addition, DQ8−/− mice produced antinuclear Abs. The B cell compartment and expression of DQ8 were normal in all the strains, although frequency of cells expressing DQ8 was less in CD4−/− mice. Decreased apoptosis was seen in CIA-susceptible DQ8 and CD8-deficient mice, indicating a defect in activation-induced cell death. These observations suggest that CD4 cells are necessary for initiation of CIA in DQ8 mice. We hypothesize that CD8+ T cells are not capable of initiating CIA in DQ8-transgenic mice but may have a regulatory/protective effect.

Rheumatoid arthritis (RA) is an autoimmune disorder characterized by synovial inflammation and erosion of bone and cartilage leading to destruction of joints. Genes in the MHC have been shown to contribute to the RA predisposition (1–2). While linkage studies have reported HLA-DRB1*0401 association with susceptibility to RA (3–7), there are reports on the role of DR4 haplotype-associated DQB1 alleles, DQB1*0301 and *0302, in RA predisposition (8–10). Published data are consistent with an involvement of T cells, especially CD4+ T cells, in pathogenesis of RA. These include 1) the infiltration of inflamed synovium with predominantly T cells, 2) improvement in joint disease manifestations of arthritis following treatment with depleting CD4 mAb, and 3) multiple oligoclonally expanded CD4 T cells within the rheumatoid joint (11–15). Recent studies suggest that RA patients carry expanded CD4 clonotypes, which are not restricted to the joint but are also found in periphery, thus indicating a systemic nature of the disease (16, 17). The observation that CD8 clonotypes are detected in synovial fluids while CD4+ clonal expansions have been found predominantly in periphery has led to the notion that CD8 T cells may play a significant role in RA pathogenesis (17, 18). T cells lacking both CD4 and CD8 surface markers have also been isolated from synovial fluid of RA patients (19). Oligoclonal expansion of T cells in RA joints is thought to be driven in part by type II collagen (CII), a putative RA-associated autoantigen, and T cells reactive to CII may be mediators of RA pathogenesis (20–22).

Collagen-induced arthritis (CIA) is an experimental model of autoimmune inflammatory polyarthritis sharing clinical and pathological features with RA (23). Susceptibility to CIA is associated with MHC class II polymorphism (H2A8 and H2A′), suggesting an important role of MHC-restricted T cell in development of disease (24). CII-specific CD4+ T cells have been reported to be fundamental in initiation and perpetuation of the disease (25, 26) and are essential for transferring arthritis into SCID mice (27). Further evidence is provided by the observations that treatment of CIA mice with either anti-TCR Ab (28) or anti-CD4 Ab (29) abrogates disease. In contrast, CII-reactive CD4+ T cells have been reported in some circumstances to protect against CIA (30). DBA/1-mCD4-deficient mice in a model of CIA were susceptible to CIA, suggesting that, in the absence of CD4+ T cells, CD8 and double-negative (DN) T cells could play a role in initiation of disease (31). While CD8+ T cells have been shown to be crucial in the onset of several experimental autoimmune disease models (32–34), the contribution of CD8+ T cells in CIA has yet to be defined.

In this study, we investigated the role of CD4+ and CD8+ T cells in initiation of CIA in transgenic mice expressing a RA-associated HLA class II molecule, DQ8 (DQA1*0301, DQB1*0302). Previous studies from our laboratory (35) have shown that Aβo-DQ8 mice elicit a vigorous CD4-mediated, DQ8-restricted cellular response following immunization using CII which progresses to a severe form of CIA. To investigate whether CD8+ T cells or any other cell can initiate CIA in Aβo-DQ8 mice in the absence of CD4 expression, we generated Aβo-DQ8 mice.
deficient in mCD4 (DQ8−CD4−/−). To delineate the role of CD8 T cells we also generated AβoDQ8 mice deficient in mCD8 molecules (DQ8−CD8−/−). While DQ8mCD4−/− mice were completely resistant to CIA, DQ8−CD8−/− mice developed a severe form of CIA with earlier onset than DQ8 mice, suggesting that CD8+ T cells may have a protective or regulatory role in arthritogenic processes.

Materials and Methods

Mice
AβoDQ8−CD4−/− and AβoDQ8−CD8−/− mice were generated by mating AβoDQ8 mice in B6/129 background with mCD4-deficient and mCD8-deficient mice (generously provided by Dr. T. Mak, University of Toronto, Toronto, Ontario, Canada), also in B6/129 background, respectively. The F1 mice were intercrossed two to four generations to generate AβoDQ8−CD4−/− and AβoDQ8−CD8−/− mice. Thus, all the mice used in this study had a similar B6/129 background. For all the groups, parental mice and negative littermates were included as controls. All mice were typed for phenotypic expression of DQ8, CD4, and CD8 molecules.

Mice of both sexes (8–12 wk of age) used in this study were bred and maintained in the pathogen-free Immunogenetics Mouse Colony at the Mayo Clinic (Rochester, MN). All mice used in this study lack endogenous class II molecules (Aβo).

Flow cytometry

Expression of HLA-DQ, H2A, CD3, CD4, CD8, pan-B, and TCR Vβ-chain was also measured. Flow cytometry was analyzed by flow cytometry using FACS IV (BD Biosciences, Mountain View, CA) as described earlier (36). Abs used for staining were IVD12 (anti-DQβ1), HB163 (anti-Aβ), GK1.5 (anti-CD4), 53.6.8 (anti-CD8), MB9-4 (anti-Vβ5.1), MB9-8 (anti-Vβ1.2), 44-22 (anti-Vβ6), F23.1 (anti-Vβ8.1,2.3), KJ-16 (anti-Vβ8.1,2), F23.2 (anti-Vβ8), and KT11 (anti-Vβ11).

Induction and evaluation of CIA

Pure native chick CII was obtained by multiple step purification described previously (37). To induce CIA, 4–to 12-wk-old transgenic mice and negative littermates were immunized with 100 μg of CII emulsified 1:1 with CFA H37Ra (100 μl; Difco, Detroit, MI) intradermally at the base of the tail. Animals received a booster of 100 μg of CII emulsified in IFA (100 μl) 28 days later. Mice were monitored for the onset and progression of CIA from 3 to 12 wk postimmunization. The arthritic severity of mice was evaluated as described previously with a grading system for each paw from 0 to 3 (24). The mean arthritic score was determined using arthritic animals only.

Histopathology

Mice were sacrificed after 10–12 wk of immunization and kidneys were extracted and snap-frozen in OCT tissue-embedding medium (Sakura Finetek, Torrance, CA). Cryostat sections were prepared, fixed, and stained with H&E. Paws from these mice were also fixed and sections were stained with H&E.

Anti-collagen Abs

Mice were bled on day 35 postimmunization, and the level of anti-mouse CII and anti-chick CII Abs in sera were determined using a standard ELISA technique as described previously (38). Briefly, microtiter plates were coated overnight with chick or mouse CII (6 μg/well in KPO4, pH 7.6) at 4°C, washed, and blocked with 1% BSA in PBS/0.05% Tween 20. Sera were added in 4-fold dilutions (1/100–1/65,000) and incubated overnight at 4°C. The plates were washed and peroxidase-conjugated goat anti-mouse IgG (Organon Teknika, West Chester, PA) was added for another overnight incubation at 4°C. After washing, O-phenylenediamine was added and the colorimetric change was measured at 410 nm. All assays were performed in duplicate and were quantitated against a standard curve obtained with a known positive sera, arbitrarily determined to equal 100 Ab units per milliliter.

Rheumatoid factor

Rheumatoid factor (RF) was measured by ELISA as previously described (39). Briefly, ELISA plates were coated with rabbit IgG overnight at 4°C. After washing, sera (1/40 dilution) were added and incubated for 45 min at room temperature and washed five times with PBS containing 0.05% Tween 20. Subsequently, wells were incubated with HRP-conjugated rabbit anti-mouse IgG (Fc specific; Pierce, Rockford, IL) or rabbit anti-mouse IgM (μ-chain specific; Pierce) for 1 h and washed. 3,3′,5,5′-Tetramethylbenzidine substrate (Sigma-Aldrich, St. Louis, MO) was added, and the absorbance spectrum was determined with automated spectrophotometer (Bio-Rad, Hercules, CA). Sera from MRL/lpr (39) and B6 mice were used as positive and negative controls, respectively. Sera from the following groups of mice were also tested concurrently: DQ8 naive mice, littermates negative for transgene, B10.Q mice immunized with CII, and DQ8−CD8−/− mice immunized with only CFA.

Antinuclear Abs

Hep-2 cell line slides (Bio-Rad) were used as substrates for antinuclear Ab (ANA) detection. Sera diluted (1/100) in PBS containing 1% BSA were added, incubated for 1 h at room temperature, and washed three times in PBS for 15 min. Bound Abs were detected with FITC-coupled goat antimouse IgG (Accurate Chemical & Scientific, Westbury, NY). Stained slides were washed three times in PBS for 15 min and examined by fluorescence microscopy. Autoantibodies to ssDNA, dsDNA, and histone were determined by ELISA as previously described (40).

In vitro T cell proliferation

Mice were immunized with 200 μg of CII emulsified 1:1 in CFA (Difco) intradermally at the base of the tail and one hind footpad. Ten days postimmunization, draining popliteal, caudal, and lumbar lymph nodes were removed and prepared for in vitro culture. A total of 1 × 106 lymph node cells (LNCs) were challenged by adding 100 μl of medium (negative control), Con A (20 μg/ml, positive control), and native collagen (50 μg/ml). Cells were plated (10 6 lymph node cells/well) in 24-well plates. After 24 h, supernatant fluids were collected and assayed for IFN-γ, IL-4, and IL-10. For induction experiments, culture supernatants containing mAb (25 μg/ml) Ab (H9262) or L12 (anti-HLA−DQ), or Lyt2 (anti-CD8) was added to the cells challenged in vitro with CII at 50 μg/ml. The cells were incubated for 48 h at 37°C. During the last 18 h the cells were pulsed with [3H]thymidine and the tritium incorporation was determined by liquid scintillation counting. Results are calculated as cpm (mean cpm of triplicate cultures containing Ag ~ mean cpm of medium).

Measurement of cytokines

Capture ELISA was done for measuring cytokines IFN-γ and IL-4 (from Genzyme, Cambridge, MA), and TNF-α, IL-10, and IL-6 (from BD Pharmingen, San Diego, CA) in culture supernatants using commercial kits.

Cell division and cytokine production by CD3+ CD4− CD8− cells

Mice were sacrificed on days 7, 14, and 21 after immunization with CII. For every experiment, pooled LNCs from two to three mice were collected and stained with conjugated Abs CD3-FITC, CD4-PE, CD8-PE, and NK1.1-PE (from BD Pharmingen) according to the manufacturer’s instructions and then sorted by FACS IV (BD Biosciences) for FITC+PE− cells. CD3+ DN cells were collected from both naive and immunized mice and cultured for further analysis. For cell division, an equal number of CD3+ DN cells were stained with cell division tracking dye, CFSE (41), and cultured in vitro for 72–96 h with or without CII. Culture supernatants were collected and assessed for cytokines.

AICD

To study the sensitivity of the proliferating cells to CII-induced activation-induced cell death (AICD), CD3+ cells were stained with annexin V conjugated with FITC after in vitro stimulation with CII and analyzed by FACS.

Statistical analysis

Difference in incidence of arthritis between groups was analyzed using the χ2 test with Yates’ correction. Ab levels and means scores for arthritic mice were compared using Student’s t test. Differences in T cell types (CD3+ DN, NK1.1) between groups was analyzed using the χ2 test.

Results

CD4-deficient mice are resistant to CIA

Transgenic HLA-DQ8 and DQ8−CD4−/− mice were immunized with CII and followed for onset and progression of arthritis for 12 wk. The DQ8 mCD4−/− mice did not show any evidence of arthritis either phenotypically or histologically, although 70% of DQ8 mice exhibited severe arthritis. There was no evidence of disease or infiltration of mononuclear cells in the synovia of...
In addition, while all the immunized DQ8 mice produced anti-CII Abs, none of the DQ8-CD4− mice produced anti-CII Abs, although the B cell numbers in CD4-deficient and DQ8 mice are comparable (50 and 56%, respectively; mean values from 10 mice in each strain).

**Increased susceptibility to the development of CIA and autoantibodies in CD8-deficient DQ8-transgenic mice**

Both DQ8 and DQ8-CD8−/− mice were immunized with CII and followed for development of arthritis. Both strains developed severe arthritis, although a higher incidence (70 vs 95%, respectively; p < 0.05) and an earlier onset (mean ± SD onset: 41 ± 12 vs 35 ± 10 days, respectively) of arthritis was detected in the latter group (Fig. 2A). A more severe disease was observed in CD8-deficient mice compared with DQ8 mice (p < 0.05) (Fig. 2B). To determine the Ab responses to CII, sera were collected and Abs to CII and mouse CII (MsII) were assayed by ELISA. All mice produced anti-CII and anti-MsII Abs, although the levels were significantly higher in the DQ8-CD8−/− mice (p < 0.0008) (Fig. 2C). Mice immunized with adjuvant alone produced no detectable anti-collagen Abs (data not shown).

**Transgenic CIA-susceptible mice produce RF**

To further determine the clinical relevance of this animal model and the fact that these mice carry human gene, sera from these mice were tested for the presence of RF. The result showed that both DQ8- and DQ8-CD8−/−-immunized produced IgM and IgG RF. The incidence and level of IgM-RF were not significantly different between DQ8-CD8−/− (74%, 25 of 34) and DQ8 (67%, 10 of 15) mice. However, more DQ8-CD8−/− mice were positive for IgG-RF (28 of 34) than DQ8 (6 of 15, p < 0.004) mice. Moreover, the levels of IgG-RF were significantly higher in DQ8-CD8−/− compared with DQ8 mice (p < 0.005) (Fig. 3). In contrast, none of the naïve DQ8, DQ8-CD4−/−, transgene negative littersmates, Aβo, and DQ8-CD8−/−-transgenic mice immunized with adjuvant alone produced a detectable level of RF. CIA-susceptible B10.Q mice also failed to develop RF following immunization with CII (data not shown).

**ANAs are present in CD8-deficient mice**

Sera obtained at day 35 postimmunization with CII or adjuvant were used to study ANAs. All DQ8-CD8−/− sera (35 of 35) tested positive for ANAs with homogenous pattern while none of the DQ8 and DQ8-CD8−/− sera elicited APA staining (Fig. 4). In addition, while DQ8 and DQ8-CD4−/− mice did not produce detectable levels of ssDNA, dsDNA, or anti-histone Abs, 66% of DQ8-CD8−/− mice produced anti-histone Abs and 43% of DQ8-CD8−/− mice produced anti-dsDNA Abs (Fig. 4). However, none of the mice positive for ANAs developed proteinuria and glomerulonephritis (data not shown).

**CD4 T cells are required for T cell response to CII**

LNCs were harvested from mice primed with CII and cultured in vitro in the presence or absence of collagen. DQ8 and DQ8-CD8−/− mice gave a robust response to immunizing collagen (Fig. 5A). Inhibition assays using specific Abs demonstrated that the response to CII in these mice was mediated by CD4+ T cells and restricted by DQ8 molecules (Fig. 5B). DQ8-CD4−/− mice mounted minimal response to CII in vitro, thus indicating that CD4 T cells are required to initiate response against CII.
Analysis of T cell types

To determine whether there was any alteration in T cell populations in our CD4 and CD8 knockout mice, we analyzed the number of different CD3+ T cell populations. Total numbers of CD3+ cells were decreased in DQ8-CD4-/- and CD8-/- mice by ~15 and 5%, respectively, when compared with DQ8 mice (Fig. 6). Of the CD3+ cells, ~80% were positive for CD4 in DQ8-CD4-/- mice and 90% were positive for CD8 in DQ8-CD4-/- mice, 20 and 10% being CD3+ DN, respectively. Expression of DQ8 was similar in all the transgenics as observed by FACS analysis, although the number of spleen cells positive for DQ molecule was lower in CD4-deficient mice than in the other two strains (Fig. 7). The number of cells positive for CD5 and CD40 were lower in DQ8-CD4-/- mice compared with DQ8 and DQ8-CD8-/- mice, although differences reached statistical significance only for CD5 (DQ8 vs DQ8-CD4-/-, p < 0.01; DQ8-CD4-/- vs DQ8-CD8-/-, p < 0.05). Interestingly, activation marker CD69 was seen with similar frequency in primed mice of all three strains.

We also investigated the Vβ usage by T cells selected by the transgenics. All the strains represented most of the Vβ, although the DQ8-CD4-/- had a decreased frequency of all the Vβ that reflects the lower number of total CD3 T cells (data not shown).

Role of CD3+ DN T cells in arthritis

A significant role of CD3+ DN T cells has been suggested for initiation of CIA (29). To determine the role of these cells in initiation/progression of arthritis, we studied CD3+ DN cells in all mice included in the study. Cells were sorted for CD3+CD4+ CD8- T cells by FACS. Characterization of CD3+ T cells showed an increased number of CD4 and DN cells in DQ8-CD8-/- mice compared with the DQ8 mice. DQ8-CD4-/- mice had more CD8- T cells and CD3+ DN cells than did DQ8 mice. However, CD3+ DN cells in DQ8-CD4-/- mice were significantly lower in number than those in DQ8-CD8-/- mice. Similar results were observed for naive and immunized mice (Table I). Moreover, the single-positive cells decreased in LNCs, while a concomitant increase in CD3+ DN T cells up to 14 days postimmunization with CIA was seen. After 21 days of immunization, CD3+ DN T cells decreased in all mice except DQ8-CD8-/-, in which they were persistently elevated and comprised 35–45% of CD3+ cells.
We further analyzed the number of CD3\(^+\) NK1.1\(^+\) cells in these mice. Interestingly, both DQ8 and DQ8\(\times\)CD8\(^{-/-}\) mice had higher number of these cells (10\%) compared with DQ8\(\times\)CD4\(^{-/-}\) (3\%), although the difference did not reach statistical significance. NK1.1\(^+\) cells increased in number up to 2 wk after immunization with a subsequent decrease.

**FIGURE 6.** Characterization of CD3\(^+\) T cells. Splenic cells were purified, labeled with conjugated Abs specific for CD4, CD8, and CD3, and analyzed by FACS. For DN cells, CD3\(^+\) cells were gated and dot blots were generated by plotting CD4 and CD8 on the x and y axes, respectively. The circled area shows CD3\(^+\) DN cells in each strain.

**FIGURE 7.** Splenic cells were labeled with MHC class II-specific mAbs and detected with a specific FITC-conjugated mAbs. The data shown were obtained from spleen cells pooled from two mice per strain and represented at least three separate assays. Costimulatory molecules CD40 and CD5 and activation marker CD69 were analyzed from purified spleen cells after labeling with specific mAbs.
Susceptible mice have a defect in AICD

To study the kinetics of cell division, splenic CD3\(^+\) and CD3\(^+\) DN T cells (sorted from LNCs) were isolated from primed mice at days 7, 14, and 21, and challenged in vitro with CII after staining them with CFSE. Higher numbers of dividing CD3\(^+\) T cells at day 14 were observed in DQ8 and DQ8\(\text{CD}8^{-}/-\) mice as compared with DQ8\(\text{CD}4^{-}/-\) mice after 48 h in response to CII (Fig. 8A). A similar phenomenon was seen in 7- and 21-day primed mice (data not shown). CD3\(^+\) DN T cells isolated from mice after 14 days of immunization also showed a higher number of cells undergoing multiple divisions in DQ8\(\text{CD}8^{-}/-\) and DQ8 mice (80 and 60\%, respectively), whereas only 45\% cells underwent division in CD4-deficient mice (Fig. 8B). Characterization of the CD3\(^+\) DN cells showed a high number of cells being positive for B220. In DQ8\(\text{CD}8^{-}/-\) mice, the CD3\(^+\)B220\(^+\) DN cells proliferated actively after immunization. In contrast, there was no significant increase in proliferation of CD3\(^+\)B220\(^+\) DN cells after immunization in the other two strains (Fig. 8C).

We further investigated whether there is a defect in AICD in mice susceptible to develop arthritis. Splenic cells and LNCs were isolated from all the primed transgenic mice and cultured in the lymph node CD3\(^+\) T (sorted from LNCs) cells were isolated from primed mice at days 7, 14, and 21 postimmunization with CII and stained with conjugated Abs as above and CFSE probe. Higher numbers of dividing CD3\(^+\) T cells at day 14 were observed in DQ8 and DQ8\(\text{CD}8^{-}/-\) mice compared with DQ8\(\text{CD}4^{-}/-\) mice, although some IL-4 was produced. Both DQ8 and DQ8\(\text{CD}8^{-}/-\) mice had higher Bcl2, with lower caspase-3 levels compared with those of CIA-susceptible mice. Seventy percent of DN cells isolated from all the primed transgenic mice and cultured in vitro with CII after staining them with CFSE showed a higher number of cells undergoing multiple divisions in DQ8\(\text{CD}8^{-}/-\) and DQ8 mice (80 and 60\%, respectively), whereas only 45\% cells underwent division in CD4-deficient mice (Fig. 8B). Characterization of the CD3\(^+\) DN cells showed a high number of cells being positive for B220. In DQ8\(\text{CD}8^{-}/-\) mice, the CD3\(^+\)B220\(^+\) DN cells proliferated actively after immunization. In contrast, there was no significant increase in proliferation of CD3\(^+\)B220\(^+\) DN cells after immunization in the other two strains (Fig. 8C).

We further investigated whether there is a defect in AICD in mice susceptible to develop arthritis. Splenic cells and LNCs were isolated from all the primed transgenic mice and cultured in the lymph node CD3\(^+\) T (sorted from LNCs) cells were isolated from primed mice at days 7, 14, and 21 postimmunization with CII and stained with conjugated Abs as above and CFSE probe. Higher numbers of dividing CD3\(^+\) T cells at day 14 were observed in DQ8 and DQ8\(\text{CD}8^{-}/-\) mice compared with DQ8\(\text{CD}4^{-}/-\) mice, although some IL-4 was produced. Both DQ8 and DQ8\(\text{CD}8^{-}/-\) mice had higher Bcl2, with lower caspase-3 levels compared with those of CIA-susceptible mice. Seventy percent of DN cells isolated from all the primed transgenic mice and cultured in vitro with CII after staining them with CFSE showed a higher number of cells undergoing multiple divisions in DQ8\(\text{CD}8^{-}/-\) and DQ8 mice (80 and 60\%, respectively), whereas only 45\% cells underwent division in CD4-deficient mice (Fig. 8B). Characterization of the CD3\(^+\) DN cells showed a high number of cells being positive for B220. In DQ8\(\text{CD}8^{-}/-\) mice, the CD3\(^+\)B220\(^+\) DN cells proliferated actively after immunization. In contrast, there was no significant increase in proliferation of CD3\(^+\)B220\(^+\) DN cells after immunization in the other two strains (Fig. 8C).

To study the kinetics of cell division, splenic CD3\(^+\) and CD3\(^+\) DN T cells (sorted from LNCs) were isolated from primed mice at days 7, 14, and 21, and challenged in vitro with CII after staining them with CFSE. Higher numbers of dividing CD3\(^+\) T cells at day 14 were observed in DQ8 and DQ8\(\text{CD}8^{-}/-\) mice as compared with DQ8\(\text{CD}4^{-}/-\) mice after 48 h in response to CII (Fig. 8A). A similar phenomenon was seen in 7- and 21-day primed mice (data not shown). CD3\(^+\) DN T cells isolated from mice after 14 days of immunization also showed a higher number of cells undergoing multiple divisions in DQ8\(\text{CD}8^{-}/-\) and DQ8 mice (80 and 60\%, respectively), whereas only 45\% cells underwent division in CD4-deficient mice (Fig. 8B). Characterization of the CD3\(^+\) DN cells showed a high number of cells being positive for B220. In DQ8\(\text{CD}8^{-}/-\) mice, the CD3\(^+\)B220\(^+\) DN cells proliferated actively after immunization. In contrast, there was no significant increase in proliferation of CD3\(^+\)B220\(^+\) DN cells after immunization in the other two strains (Fig. 8C).

We further investigated whether there is a defect in AICD in mice susceptible to develop arthritis. Splenic cells and LNCs were isolated from all the primed transgenic mice and cultured in the lymph node CD3\(^+\) T (sorted from LNCs) cells were isolated from primed mice at days 7, 14, and 21 postimmunization with CII and stained with conjugated Abs as above and CFSE probe. Higher numbers of dividing CD3\(^+\) T cells at day 14 were observed in DQ8 and DQ8\(\text{CD}8^{-}/-\) mice compared with DQ8\(\text{CD}4^{-}/-\) mice, although some IL-4 was produced. Both DQ8 and DQ8\(\text{CD}8^{-}/-\) mice had higher Bcl2, with lower caspase-3 levels compared with those of CIA-susceptible mice. Seventy percent of DN cells isolated from all the primed transgenic mice and cultured in vitro with CII after staining them with CFSE showed a higher number of cells undergoing multiple divisions in DQ8\(\text{CD}8^{-}/-\) and DQ8 mice (80 and 60\%, respectively), whereas only 45\% cells underwent division in CD4-deficient mice (Fig. 8B). Characterization of the CD3\(^+\) DN cells showed a high number of cells being positive for B220. In DQ8\(\text{CD}8^{-}/-\) mice, the CD3\(^+\)B220\(^+\) DN cells proliferated actively after immunization. In contrast, there was no significant increase in proliferation of CD3\(^+\)B220\(^+\) DN cells after immunization in the other two strains (Fig. 8C).

We further investigated whether there is a defect in AICD in mice susceptible to develop arthritis. Splenic cells and LNCs were isolated from all the primed transgenic mice and cultured in the lymph node CD3\(^+\) T (sorted from LNCs) cells were isolated from primed mice at days 7, 14, and 21 postimmunization with CII and stained with conjugated Abs as above and CFSE probe. Higher numbers of dividing CD3\(^+\) T cells at day 14 were observed in DQ8 and DQ8\(\text{CD}8^{-}/-\) mice compared with DQ8\(\text{CD}4^{-}/-\) mice, although some IL-4 was produced. Both DQ8\(\text{CD}4^{-}/-\) and DQ8\(\text{CD}8^{-}/-\) mice produced IL-6, although there was a consistent trend toward increased levels of IL-6 production in DQ8\(\text{CD}8^{-}/-\) compared with DQ8\(\text{CD}4^{-}/-\) and DQ8 mice. However, differences did not reach statistical significance (Fig. 10B). There was a late production of IL-10 in DQ8\(\text{CD}4^{-}/-\) mice after 21 days of immunization and 96 h in vitro culture only, while in DQ8 and CD8\(\text{CD}8^{-}/-\) mice IL-10 was detected after 7 days of immunization. CD3\(^+\) DN cells did not produce IFN-\(\gamma\) in all mouse strains, although a large amount of TNF-\(\alpha\) was produced in response to

![FIGURE 8. CFSE-labeled LNCs were cultured in vitro with CII. On day 3, CFSE profiles of activated cells were determined using flow cytometry. A. Cells were stained with CD3-FITC and CFSE profiles were generated from CD3\(^+\) gated cells. DQ8 (solid line) and DQ8\(\text{CD}8^{-}/-\) (dashed line) cells proliferated at comparable rates, whereas DQ8\(\text{CD}4^{-}/-\) (dotted line) cells proliferated at a slower rate. B. CD3\(^+\) DN cells were sorted from LNCs of primed mice. Equal numbers of CFSE-labeled cells were cultured as above and CFSE profiles were determined using flow cytometry. CD3\(^+\) DN cells from DQ8\(\text{CD}8^{-}/-\) mice underwent more divisions than the other two strains. C. Spleen cells were stained for CD3, CD4, CD8, and B220. CD3\(^+\) DN T cells were gated and analyzed for the presence of B220. Bars denote the percentage of CD3\(^+\)B220\(^+\) DN cells of the total CD3\(^+\) DN T cells in each strain.](http://www.jimmunol.org/Download)
CII in DQ8-CD4−/− mice. In DQ8 and DQ8-CD8−/− mice, CD3−DN cells produced large amounts of IL-10 (Fig. 10C).

Discussion
CIA has been a murine model for human RA for well over 20 years. However, there have been several reservations concerning its clinical and pathological similarities to human disease. The main concern with this model has been that mice do not produce RF, one of the major features of the human disease. Using human class II DQ8 transgenics in conjunction with CD4 and CD8 knockouts we have collected data further establishing the relevance of these mice to investigations of human RA. A novel feature of transgenics expressing the human DQ8 susceptibility allele is the detection of RF in the sera of C1A+ mice. This study marks the first report of RF in CIA mice expressing RA-linked human MHC molecules, thereby demonstrating the HLA-transgenic mice as a valuable model to study human disease. DQ8-transgenic mice express a normal repertoire of Vγ8 TCRs and develop severe CIA (35), indicating that DQ8 molecule can interact efficiently with mCD4. DQ8 is a promiscuous molecule that can bind many antigenic peptides with low affinity. Thus, one plausible explanation for autoimmune responses in DQ8-transgenic mice could be thymic selection of potential autoreactive T cells by DQ8 molecules which have escaped central tolerance.

Our observations indicate an important role of CD4 T cells in initiation of CIA and CD8 T cells in regulation of disease. The scenario in the development of arthritis in transgenic mice can be compared with that of RA in human. Both require presentation of arthritogenic epitope by HLA class II molecules to CD4 T cells, leading to proliferation of autoreactive T cells and production of RF by B cells subsequently leading to joint pathology. Using CD8 knockout mice we have associated CD8 T cells with an immunoregulatory role in CIA, as absence of CD8 leads to production of ANAs and more severe disease. A similar phenomenon can be envisaged in RA, where production of autoantibodies like RF and ANAs could be related to the functional status of CD8 T cells. However, susceptibility conferred by class II alleles might be modulated by MHC class I polymorphism. Indeed, it has been shown that CII can be recognized by class I-restricted T cells (31, 42). In the CIA model, contrasting observations have been reported on the role of CD8 T cells (42, 43). Depletion of CD8 T cells has been shown to suppress or lower incidence of arthritis in the CIA model (31, 44), while other studies have reported no role of CD8 T cells (42) or immunomodulation of arthritis by CD8 T cells (43). In some autoimmune diseases, CD8+ T cells have been shown to transfer disease from affected to naive mice, but they require the help of CD4 T cells (34). In our study, DQ8-CD8−/− mice get severe arthritis with earlier onset and produce high amounts of proinflammatory cytokines (IFN-γ and TNF-α) as well as immunoregulatory cytokines (IL-6 and IL-10) in response to CII. The present data provide evidence that CD8 T cells modulate disease, in part, by down-regulation of potentially pathogenic Ag-specific Th1 cells in vivo. This could lead to skewing of the cytokine profile and decreased production of autoantibodies. In vivo depletion of CD8 T cells in DQ8-transgenic mice can provide further evidence of immunomodulatory role of CD8+ T cells.

Most of the studies in RA and animal models of arthritis have implicated CD4+ T cells to be primarily responsible for disease activity. However, class II-restricted response to T cell-dependent Ag and production of cytokines by CD3+ DN T cells upon activation in mouse models and humans indicate an immunoregulatory role of these cells in vivo (45–47). In the CIA model, CD3+ DN T cells have been shown to play an important role in disease pathogenesis (31). Our results showed an increased number of CD3+ DN T cells in DQ8-CD8−/− mice compared with DQ8-CD4−/− mice. Also, CD3+ DN cells proliferated in response to CII indicating a role in pathogenesis of arthritis, although they may not be involved in initiation of the disease. Higher proliferation of CD3+ DN T cells in DQ8-CD8−/− mice compared with other two strains could be another factor responsible for increased severity in DQ8-CD8−/− mice. An increased number of CD3+ B220+ DN T cells in DQ8-CD8−/− mice corroborates recent data showing that CD4+ CD8− T cells survive chronic stimulation and further suggests that these cells might persist as Ag-specific DN B220+ T cells (48). Our results also showed that CD3+ DN cells produced TNF-α in vitro in response to CII; however, in the DQ8-CD4−/− mice, other cell types were not able to mount optimum specific
response and direct role in initiation/pathogenesis but might be involved in progress of disease. Osshima et al. (50) demonstrated that IL-6 knockout mice develop a more modest form of CIA, which supports the notion that IL-6 may not be involved directly in disease pathogenesis.

Oligoclonal CD4+ T cells from arthritic patients have been shown to have defects in apoptotic pathways (51). CD4+ T cells from CIA-resistant DQ8 CD+− mice showed enhanced apoptosis compared with susceptible DQ8 and DQ8 CD+− mice. Decreased apoptosis in susceptible mice was confirmed by poor cleavage of caspase-3, a hallmark of apoptosis. Increased expression of antiapoptotic protein, Bcl2, in CIA-susceptible mice correlates with decreased apoptosis in RA patients. This indicates a possible defect in AICD in mice developing arthritis. Activation of CD3+ T cells with subsequent resistance to apoptosis may contribute to CIA pathology.

Our data bring forth the following points: 1) transgenic mice expressing the RA-associated HLA-DQ8 gene develop CIA mediated by CD4 T cells and also produce RF similar to RA, 2) CD3+ DN T cells can undergo chronic stimulation and may persist as Ag-specific T cells, 3) expansion following exposure to Ag as well as a defect in AICD might lead to pathology seen in RA and CIA, and 4) CD8 T cells play a regulatory role, as their absence leads to production of higher levels of autoantibodies.

Acknowledgments

We thank Dr. Chris Kcro for critical comments on the manuscript, Julie Hanson and Tad Trejo for maintaining transgenic mice, and Michele Smart for screening the transgenic mice.

References


