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Intrinsic Differences in Donor CD4 T Cell IL-2 Production Influence Severity of Parent-into-F1 Murine Lupus by Skewing the Immune Response Either toward Help for B Cells and a Sustained Autoantibody Response or toward Help for CD8 T Cells and a Downregulatory Th1 Response

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Intrinsic Differences in Donor CD4 T Cell IL-2 Production Influence Severity of Parent-into-F1 Murine Lupus by Skewing the Immune Response Either toward Help for B Cells and a Sustained Autoantibody Response or toward Help for CD8 T Cells and a Downregulatory Th1 Response

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Using the parent-into-F1 model of induced lupus and (C57BL/6 × DBA2) F1 mice as hosts, we compared the inherent lupus-inducing properties of the two parental strain CD4 T cells. To control for donor CD4 recognition of alloantigen, we used H-2^d identical DBA/2 and B10.D2 donor T cells. We demonstrate that these two normal, nonlupus-prone parental strains exhibit two different T cell activation pathways in vivo. B10.D2 CD4 T cells induce a strong Th1/CMI pathway that is characterized by IL-2/IFN- γ expression, help for CD8 CTLs, and skewing of dendritic cell (DC) subsets toward CD8a DCs, coupled with reduced CD4 T follicular helper cells and transient B cell help. In contrast, DBA/2 CD4 T cells exhibit a reciprocal, lupus-inducing pathway that is characterized by poor IL-2/IFN- γ expression, poor help for CD8 CTLs, and skewing of DC subsets toward plasmacytoid DCs, coupled with greater CD4 T follicular helper cells, prolonged B cell activation, autoantibody formation, and lupus-like renal disease. Additionally, two distinct in vivo splenic gene-expression signatures were induced. In vitro analysis of TCR signaling revealed defective DBA CD4 T cell induction of NF- κ B, reduced degradation of I κ B α , and increased expression of the NF- κ B regulator A20. Thus, attenuated NF- κ B signaling may lead to diminished IL-2 production by DBA CD4 T cells. These results indicate that intrinsic differences in donor CD4 IL-2 production and subsequent immune skewing could contribute to lupus susceptibility in humans. Therapeutic efforts to skew immune function away from excessive help for B cells and toward help for CTLs may be beneficial. *The Journal of Immunology*, 2015, 195: 2985–3000.

Systemic lupus erythematosus (lupus) is an immune-mediated, multisystem disease that is characterized by pathogenic autoantibodies against nuclear Ags (1). CD4 T cells are necessary and sufficient for lupus induction and are central in driving B cell production of autoantibodies in human and murine lupus. CD4 T follicular helper (Tfh) cells provide help

(e.g., IL-21) to autoreactive B cells in the germinal center (GC) (2, 3), and the resulting pathogenic IgG autoantibodies exhibit the hallmarks of a normal T cell–driven Ag-driven response (e.g., class switching, somatic mutation, and affinity maturation) (4–8). Disease expression is modified by genetic, hormonal, and environmental factors (9). A major gap in our knowledge is the mechanism by which T cell tolerance is lost and lupus ensues.

A useful model for studying the role of Ag-specific T cells in lupus pathogenesis is the parent-into-F1 (p→F1) model of chronic graft-versus-host disease (GVHD) (reviewed in Ref. 10) in which a loss of T cell tolerance is experimentally induced in normal mice, and lupus ensues. Following the transfer of homozygous parental strain CD4 T cells into unirradiated semiallogeneic nonlupus-prone F1 mice, donor CD4 T cells recognize host allogeneic MHC class II-bearing cells, resulting in the expansion of host dendritic cells (DCs), cognate help to B cells, autoantibody production, and a -lupus-like phenotype. Cotransfer of parental CD4 and CD8 T cells results in an additional phase of donor CD4 help for donor CD8 T cells specific for host allogeneic MHC class I, which then mature into CTL effectors and eliminate host lymphocytes. Thus, a selective loss of CD4 T cell tolerance results in an autoimmune, stimulatory, lupus-like phenotype. In contrast, a loss of both CD4 and CD8 T cell tolerance results in an acute GVHD phenotype manifested by a CTL-mediated immune deficiency (similar to human acute GVHD) that aborts the progression to lupus-like disease.

Interestingly, the degree of similarity between CD4-driven chronic GVHD in this model and human lupus varies with the donor and host strains used. Host genetics contribute to lupus severity in chronic GVHD (11). However, a role for donor strain

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Abbreviations used in this article: B6, C57BL/6; B10.D2, B10.D2-*H2^d* H2-T18^c/oSnJ; BDF1, B6D2F1; DBA, DBA/2DBA/2; DC, dendritic cell; GC, germinal center; GrB, granzyme B; GVHD, graft-versus-host disease; HVG, host-versus-graft; pDC, plasmacytoid DC; p→F1, parent-into-F1; pfp, perforin; SLEC, short-lived effector CD8 CTL; Tfh, T follicular helper.

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genetics has not been fully evaluated. Studies using the B6D2F1 (BDF1) strain as host are consistent with this possibility. Specifically, transfer of parental strain DBA/2 (DBA) splenocytes into BDF1 mice induces a disease that strongly resembles human lupus and is characterized by lupus-specific autoantibodies (anti-dsDNA, anti-PARP), lupus-like renal disease progressing to nephrotic syndrome, lupus-like Ig and C' deposition in the skin, positive Coombs test, and a female predilection (10, 12–16). As with human lupus, organ-specific autoantibodies are not observed in chronic GVHD mice (15). In contrast, chronic GVHD induced in BDF1 hosts using the opposite parent [i.e., C57BL/6 (B6) CD4 T cells] results in transient CD4 T cell-driven B cell hyperactivity with mild renal disease without sex differences (17). A similar mild transient lupus is seen with B6 donors transferred into MHC disparate non-F1 hosts (i.e., B6→Bm12) (16), suggesting that B6 CD4 T cells inherently induce only mild lupus.

Similarly, acute GVHD in BDF1 mice exhibits donor strain variability. Transfer of unfractionated B6 donor splenocytes into BDF1 mice (B6→F1) induces a strong Th1/CMI response at days 7–10 (10, 18–20) as evidenced by significant expansion of donor CD8 T cells with effector phenotype (pfp⁺, GrB⁺), expansion of CTL-promoting CD11c⁺ DCs, and a 2–3-fold log increase in serum IFN- γ . Engrafted B6 donor CD8 effector CTLs are specific for host MHC class I (H-2^d) and use perforin (pfp) and FasL pathways to eliminate host lymphocytes. Host B cells are highly susceptible to elimination and are profoundly reduced by 2 wk (~10% of control F1). In contrast, transfer of unfractionated DBA splenocytes into BDF1 mice (DBA→F1) is associated with a failure of donor DBA CD8 CTL maturation and of host CD11c DC expansion, coupled with only modest increases in serum IFN- γ . In the absence of donor CD8 CTL effectors, host B cells are expanded significantly (~150–200% of control F1) by day 14 as a result of donor CD4 T cell cognate help to F1 B cells following recognition of host allogeneic MHC class II (I-A^b) (10, 18–20).

Together, these observations support the idea that B6 and DBA CD4 T cells exhibit strain differences in their lupus-inducing proclivity and that this difference may be reciprocally related to their ability to help CD8 CTLs. To further explore this possibility, we compared the intrinsic CD8-promoting versus lupus-promoting properties of C57 black background or DBA background CD4 T cells. Our results indicate that these two otherwise normal (nonlupus-prone) donor strains induce two completely different activation pathways consisting of either a strong Th1/CMI-promoting pathway that is characterized by strong help for CD8 CTLs and transient help for B cells or a lupus pathway that is characterized by a poor Th1/CMI response and poor help for CD8 CTLs but strong and sustained help for B cells. These distinct phenotypic outcomes may be functionally related to inherent differences in TCR signaling pathways that drive IL-2 production in CD4 T cells. In particular, DBA T cells exhibit reduced TCR activation of NF- κ B, which may be due to increased expression of the NF- κ B modulator A20 in TCR-stimulated DBA T cells.

Materials and Methods

Mice

Six- to eight-week-old female DBA/2 (H-2^d), B6 J(H-2^b), and B10.D2-Hc^o H2^d H2-T18c^o/oSnJ (B10.D2) (H-2^d) donor mice and female BDF1 (H-2^{b/d}) recipient host mice were purchased from The Jackson Laboratory (Bar Harbor, ME). All animal procedures were preapproved by the Institutional Animal Care and Use Committee at the Uniformed Services University of Health Sciences.

Induction of GVHD

Single-cell suspensions of donor strain splenocytes were prepared as described (21) and transferred into age-matched BDF1 hosts by tail vein injection. The percentages of donor CD4 and CD8 T cell populations were analyzed by flow cytometry, and donor inoculum was adjusted prior to transfer to achieve the desired amount of donor CD4 and CD8 T cells as indicated in the text and/or respective figure legends. In some experiments, acute GVHD was induced using purified donor T cell subsets achieved through negative isolation using Dynal mouse CD4 or CD8 Negative Isolation kits (Invitrogen, Carlsbad, CA), which deplete B cells, NK cells, monocytes/macrophages, DCs, granulocytes, platelets, erythrocytes and either CD8 or CD4, respectively. Purity was confirmed by flow cytometry and was typically >95%.

Host NK depletion

F1 mice were depleted of host NK cells, as previously described (22). Briefly, mice received 200 μ g anti-NK1.1 mAb (PK136; BioLegend, San Diego, CA) i.p. 2 d prior to and 2 d following donor cell transfer. At day 7, splenic host NK1.1⁺, asialo-GM1⁺ cells in untreated mice averaged 0.3×10^6 , but they were below the limits of detection in anti-NK1.1-treated mice.

Flow cytometric analysis

Spleen cells were incubated with anti-murine Fc γ R II/III mAb, 2.4G2 for 10 min and then stained with saturating concentrations of Alexa Fluor 488-conjugated, allophycocyanin-conjugated, biotin-conjugated, PE-conjugated, FITC-conjugated, PerCPy5.5-conjugated, Alexa Fluor 647-conjugated, and Pacific Blue-conjugated mAbs against CD3, CD4, CD8, CD19, B220, H2-K^b, I-A^b, H-2K^d, I-A^d, CD11b and CD11c, CD44, CD62L, CXCR5, ICOS, PD1, CCR7, KLRG-1, GL-7, and mPDCA that were purchased from BD Biosciences (San Jose, CA), BioLegend (San Diego, CA), eBioscience (San Diego, CA), or Invitrogen. Biotinylated primary mAbs were detected using PE-Texas Red-streptavidin (BD Biosciences). Cells were fixed in 1% paraformaldehyde before reading.

Ex vivo intracellular staining for pfp, granzyme B (GrB), IFN- γ , TNF, and IL-2 was performed using Abs and reagents purchased from BD Biosciences or BioLegend, and staining was performed according to the manufacturer's instructions. Importantly, as previously described (23), there was no in vitro restimulation or use of Golgi blocking agents. Following completion of the staining protocol, cells were analyzed immediately by flow cytometry. For donor cell division studies, a CellTrace CFSE Cell Proliferation Kit was used for in vivo labeling of cells to trace multiple generations using dye dilution by flow cytometry. Donor cells were labeled according to the manufacturer's instructions prior to transfer, and host splenocytes were analyzed 3 and 4 later by flow cytometry and ModFit (Verity Software). Intracellular staining for Ki-67 was performed using a PE Mouse Anti-Human Ki-67 Set from BD Biosciences and the Foxp3 Buffer Staining Set from eBioscience, according to manufacturer's protocol. Briefly, cells were permeabilized overnight in fixation/permeabilization solution, washed in permeabilization buffer, stained with PE-conjugated mouse anti-human Ki-67 in permeabilization buffer for 30 min, washed, and analyzed immediately by flow cytometry.

Multicolor flow cytometric analyses were performed using a BD LSR II flow cytometer (BD Biosciences). The following gating strategy was used: lymphocytes were gated by forward and side scatter, and fluorescence data were collected for a minimum of 10,000 gated cells. Studies of donor T cells were performed on a minimum of 5000 cells collected using a lymphocyte gate that was positive for CD4 or CD8 and negative for MHC class I of the uninjected parent (H-2K^d). B cells were gated as positive for B220 and either positive (host origin) or negative (donor origin) for MHC class II of the uninjected parent (I-A^d). Short-lived effector CD8 CTLs (SLECs) were assessed as KLRG-1⁺, CCR7[−] gated donor CD8 T cells. Host DCs and macrophages were identified as I-A^d and CD11c⁺ or CD11b⁺, respectively, using a broad forward and side scatter gate. In Fig. 7, DCs were further gated as CD3/CD19[−], CD11c⁺ and either CD8a⁺ or mPDCA⁺.

Cytokine expression by real-time PCR

Real-time PCR was performed as described (21). Briefly, splenocytes (1×10^7) were homogenized in 1 ml RNA STAT-60 (Tel-Test, Friendswood, TX). cDNA was synthesized from mRNA using TaqMan Reverse Transcription Reagents (Applied Biosystems, Foster City, CA). Real-time PCR was performed using premade primers and probes from TaqMan Gene Expression Assays and TaqMan Universal PCR Master Mix (Applied Biosystems) for the following targets: IFN- γ , IL-10, IL-2, IL-21, IL-6, IL-4, OAS, MX-1 and IP-10, with 18s rRNA as an internal control. The

calculation of relative gene expression differences was done by the comparative $2^{-\Delta\Delta CT}$ method. The result was expressed as fold change in the experimental groups compared with uninjected BDF1 control.

In vitro IL-2 studies

For studies using unfractionated splenocytes, B6 and DBA mice were tested individually ($n = 3$ –4/group). Splenocytes were first examined by flow cytometry for CD4 T cell percentage and 0.8×10^6 CD4 T cells (4 – 5×10^6 splenocytes) were plated in 24-well flat-bottom plates and stimulated with anti-CD3 mAb ($0.25 \mu\text{g}/\text{well}$; BioLegend). In a pilot experiment, we observed that 0.25 or $0.50 \mu\text{g}$ anti-CD3 gave roughly equivalent IL-2 responses for B6, DBA, MRL $^{l/l}$, and MRL/lpr splenocytes (data not shown). For studies of purified cells, CD4 T cells were purified by negative isolation using Dynal mouse beads, as described above, from pooled age- and sex-matched B6 or DBA donors, plated at $4 \times 10^6/\text{well}$, and stimulated with anti-CD3/anti-CD28 ($0.5 \mu\text{g}/\text{ml}$) (BioLegend). Purity of CD4 T cells was 98.5%. For both studies, supernatants were harvested at 48 h, IL-2 content was determined by ELISA (R&D Systems, Minneapolis, MN), and arbitrary units were calculated from a standard curve using the TITRI program, as described (21). Cells were harvested at 48 h from separate wells and assessed for IL-2 mRNA by real-time PCR, as described above. Purified CD4 T cells also were examined for intracellular expression of IL-2 and for Foxp3, as described above.

Splenic gene expression analysis

Total RNA was isolated from individual subjects using an RNeasy Mini Kit after immediate stabilization of RNA in spleen tissue using RNAlater Reagent (both from QIAGEN, Valencia, CA). Total RNA integrity was assessed using Experion RNA HighSens Chips on an Experion Automated Electrophoresis Station (Bio-Rad, Hercules, CA). Samples with RNA integrity > 8.0 were labeled using the TotalPrep RNA Labeling Kit (Ambion, Grand Island, NY) before hybridization with MouseRef-8 v2.0 Expression BeadChips (Illumina, San Diego, CA) and imaging on an iScan Microarray Scanner (Illumina) at the University of Chicago Genomics Core. Raw data output was generated from images using the GEX module of GenomeStudio (Illumina). Raw data were preprocessed by offset background correction and quantile normalization. Nonaccurately detected BeadArray features were removed from preprocessed data for features with a detection p value > 0.1 in $\geq 7\%$ of subjects in all experimental groups. Differentially expressed transcripts were identified in the GenePattern 3.8 genomics analysis platform using the Comparative Marker Selection module (Broad Institute, Massachusetts Institute of Technology) (24, 25).

Intracellular signaling studies

CD4 T cells were purified from DBA and B6 mice using a Negative Isolation Kit (Invitrogen). T cells were stimulated by plate-bound anti-CD3/anti-CD28 for the indicated times, followed by harvest and preparation of whole-cell lysates, as previously described (26–28). Lysates were subjected to SDS-PAGE, followed by immunoblotting with Abs for I κ B α , A20, and GAPDH.

Serological studies

Mice were bled at the times indicated, and sera were tested by ELISA for the presence of IgG Abs to calf thymus DNA (Sigma-Aldrich, Atlanta, GA), as described (21).

Urine protein

Urine protein was quantitated by dipstick (Albustix; Bayer, Pittsburgh, PA).

Kidney histopathology

Kidneys were processed, stained with periodic acid–Schiff, and analyzed as previously described (12).

Statistical analysis

Statistical comparisons (t test and ANOVA) were performed using Prism 5.0 (GraphPad Software, San Diego, CA).

Results

Results following transfer of unfractionated donor splenocytes

Defective *in vivo* Th1 cytokine expression by DBA T cells. To determine the mechanisms responsible for the failure of donor DBA CD8 T cells to mature into effector CTLs in DBA→F1 mice, we ex-

amined donor and host populations at several early time points. To preclude the possibility that differences in B6 versus DBA T cells reflect differences in host alloantigen recognition, we replaced B6 donors with B10.D2 donors, a strain in which the DBA-derived H-2 complex was introgressed onto the B57BL/10Sn background. The C57BL/10 strain has a background that is highly similar to B6 mice. Importantly, H-2 d B10.D2 donors, like DBA donors, recognize F1 host H-2 b . We showed previously that acute GVHD in B10.D2→BDF1 mice strongly resembles that of B6→F1 mice (20). Thus, the chronic GVHD phenotype in DBA→F1 mice is not a consequence of an H-2 d donor recognizing a semiallogeneic H-2 b host. Importantly, the minor H-2 differences between B6 and C57BL/10 mice are not sufficient to cause a significant host-versus-graft (HVG) response, a rejection of donor T cells, or an alteration in acute GVHD phenotype (20).

F1 mice received unfractionated B10.D2 or DBA donor splenocytes adjusted to contain comparable numbers of CD4 T cells and CD8 T cells. Significant differences between these two groups were seen at days 5 and 7 after transfer. Specifically, compared with DBA→F1 mice at either day 5 or 7, donor CD4 and CD8 T cells from B10.D2→F1 mice exhibited significantly greater numbers of total cells (Fig. 1A, 1B), IFN- γ -expressing cells (Fig. 1C, 1D), and Ki-67–incorporating proliferating donor CD4 and CD8 T cells (data not shown). B10.D2→F1 mice also exhibited significantly greater numbers of activated donor CD4 and CD8 T cells (i.e., CD44 upregulation or CD62L downregulation; data not shown), although the difference was not significant at day 5. Thus, compared with DBA→F1 mice, B10.D2→F1 mice exhibited stronger donor T cell activation and a greater early Th1/CMI response, similar to that reported for B6→F1 mice (22), whereas the early Th1 response in DBA→F1 mice was significantly impaired. The similarity between B10.D2→F1 and B6→F1 mice indicates that the defective Th1 response in DBA→F1 mice is not a consequence of H-2 b host allorecognition by H-2 d donor T cells.

It was reported that host NK cells can alter lupus-like disease in DBA→F1 mice (29). To address whether the immune skewing of donor T cells seen in Fig. 1C and 1D is a primary effect of donor T cells or a secondary consequence of differential host NK function, donor T cell skewing was assessed in B6→F1 and DBA→F1 mice depleted of host NK cells. NK depletion in B6→F1 mice enhanced total donor T cell engraftment and significantly boosted the numbers of donor CD4 and CD8 T cells expressing IFN- γ and TNF (Fig. 1E–J, bar 1 versus bar 2). NK depletion in DBA→F1 mice did not significantly alter donor T cell total numbers or numbers of IFN- γ - or TNF-expressing donor T cells (Fig. 1E–J, bar 3 versus bar 4). Importantly, in NK-depleted groups, the disparity between B6→F1 and DBA→F1 mice was even greater for total donor T cell engraftment and numbers of IFN- γ - and TNF-expressing donor CD4 and CD8 T cells. Thus, host NK depletion does not reverse donor T cell immune skewing; instead, it exacerbates this effect, supporting the conclusion that the early immune skewing of donor T cells is due to intrinsic differences in donor T cells rather than secondary to host NK cells. Moreover, these results are consistent with previous work indicating that NK cells contribute to the counter-regulatory HVG response that impairs the donor T cell (particularly CD8)-mediated graft-versus-host response (22, 30). Because the donor CD8 graft-versus-host response is stronger in B6→F1 versus DBA→F1 mice (22, 30), the boosting of donor T cell engraftment is significantly greater in the absence of host NK cells.

This differential Th1/CMI response in GVHD mice was not confined to donor T cells; it also included host non-T cell populations. We examined a broad range of host non-T cell/APC

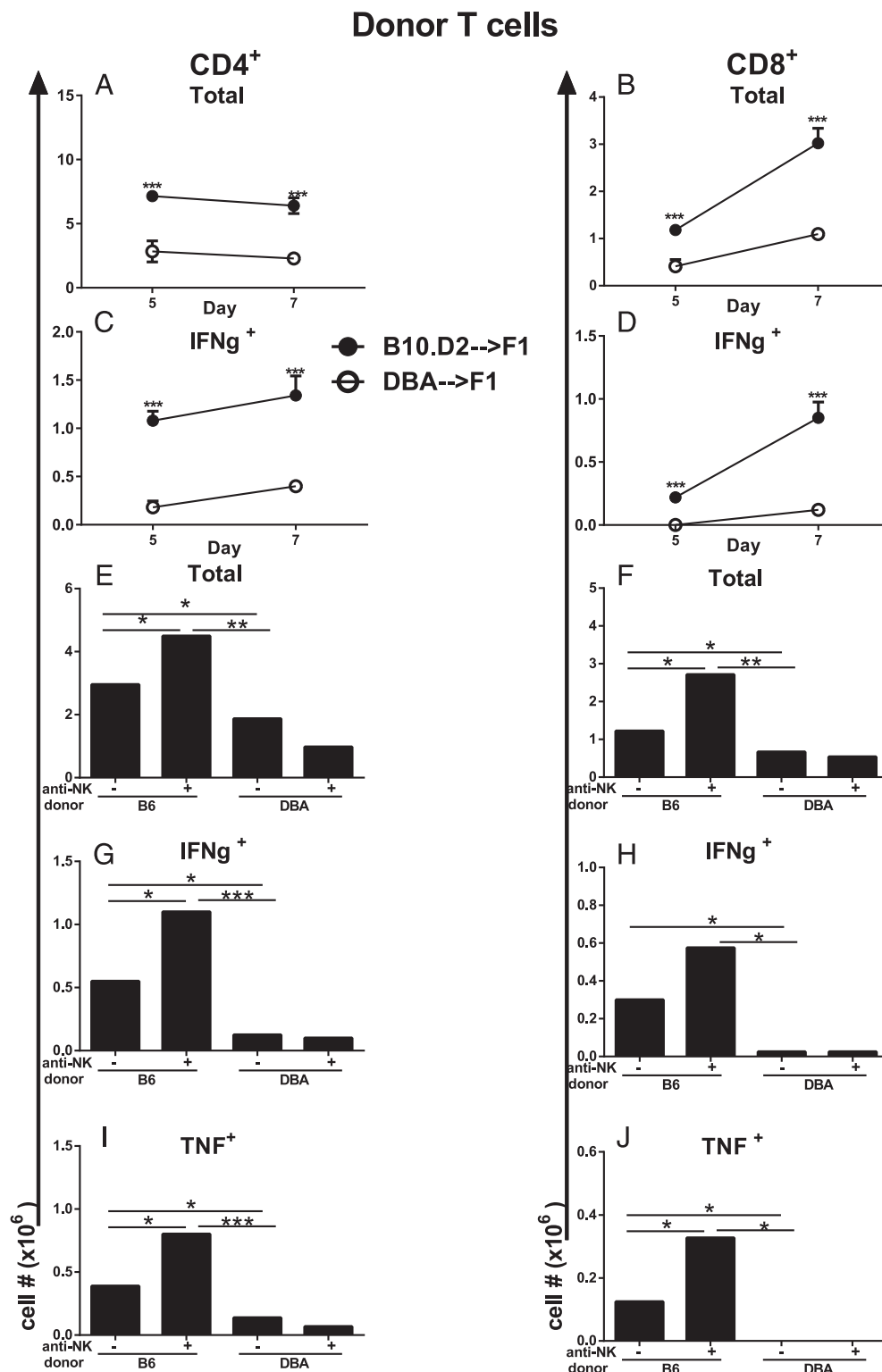


FIGURE 1. Defective in vivo Th1 cytokine expression by DBA T cells is not due to host NK function. BDF1 mice received a single injection of unfractionated splenocytes from either B10.D2 or DBA donors. On days 5 and 7 after donor cell transfer, host spleens were evaluated by flow cytometry for donor T cell numbers of CD4 T cells (**A** and **C**) and CD8 T cells (**B** and **D**) to include total numbers (**A** and **B**) and intracellular IFN- γ expression (**C** and **D**), as described in *Materials and Methods*. (**E–J**) Host F1 mice were either left untreated or were depleted of NK cells prior to receiving unfractionated B6 or DBA splenocytes, as described in *Materials and Methods*. Mice were assessed at day 7 by flow cytometry for total numbers of donor CD4 and CD8 T cells (**E** and **F**) and for intracellular expression of IFN- γ (**F** and **H**) or TNF (**I** and **J**) in donor CD4 (**G** and **I**) and CD8 (**H** and **J**) T cells, as described in *Materials and Methods*. Data are group mean \pm SE ($n = 4–5$ /group). Donor cells were examined by flow cytometry prior to transfer, and the inocula were adjusted so that each contained 5×10^6 CD8 T cells and $7.8–9.6 \times 10^6$ CD4 T cells. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

populations (CD11c⁺ or CD11b⁺). No significant differences were seen at day 5; however, by day 7, B10.D2 \rightarrow F1 mice exhibited significantly greater numbers of total CD11c⁺ or CD11b⁺ APCs

(Fig. 2A, 2B), IFN- γ -expressing APCs (Fig. 2C, 2D), and TNF-expressing APCs (Fig. 2E, 2F) compared with DBA \rightarrow F1 mice. Taken together with Fig. 1, these data confirm that B10.D2 \rightarrow F1

Host cells

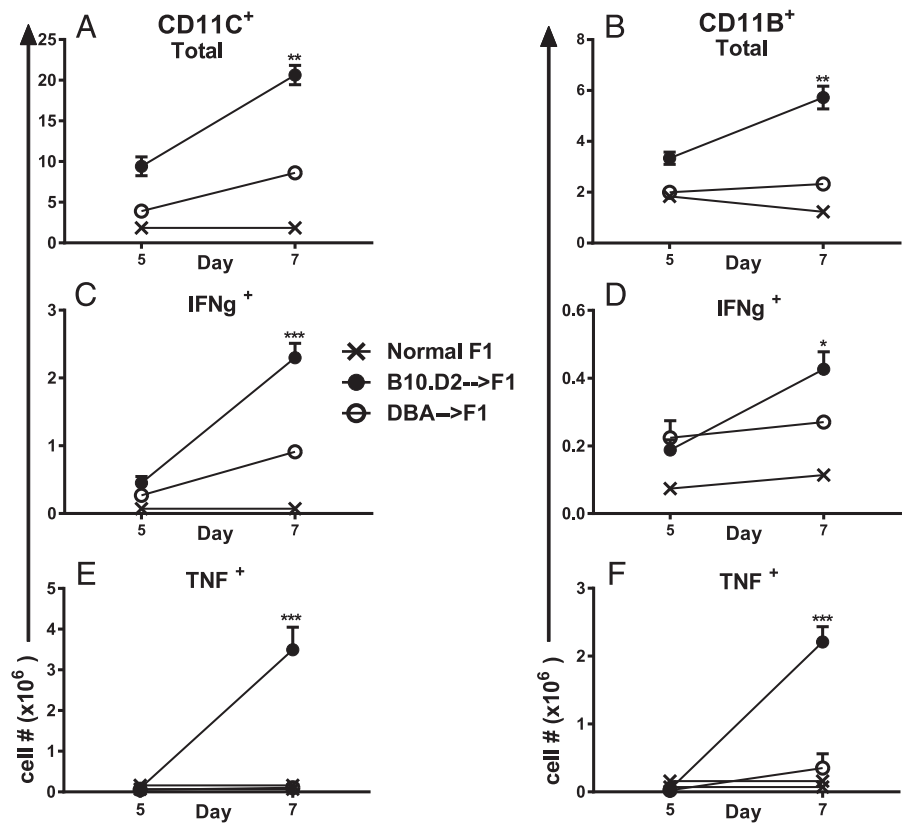


FIGURE 2. Greater production of IFN- γ and TNF expression by host APCs in B10.D2→F1 mice. The cohort described in Fig. 1A–D was also analyzed for host DC subsets by flow cytometry. Gating strategy is as described in *Materials and Methods*. CD11c⁺ cells (**A**, **C**, and **E**) and CD11b⁺ cells (**B**, **D**, and **F**), including total numbers of cells (A and B), IFN- γ -expressing cells (C and D), and TNF-expressing cells (E and F). Data are group mean \pm SE. * p < 0.05, ** p < 0.01, *** p < 0.001.

and B6→F1 mice exhibit a strong donor and host Th1/CMI response compared with DBA→F1 mice (22). Significant donor T cell differences are seen by day 5, whereas significant host differences are not seen until day 7, supporting the idea that host immune skewing is secondary to a primary intrinsic difference in donor T cells. Representative flow cytometry tracings from day 7 are shown in Supplemental Fig. 1.

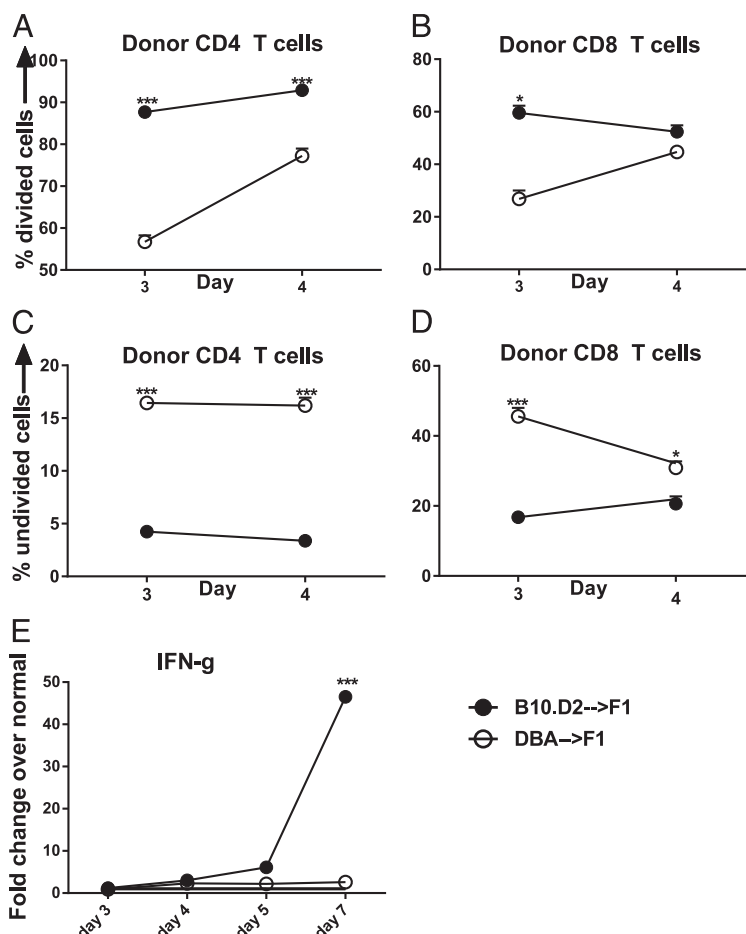
Defective initial proliferation of DBA donor T cells. To determine whether the defects in DBA T cell expansion and maturation at days 5–7 (Fig. 1) are preceded by even earlier defects, we examined initial donor T cell proliferation at days 3 and 4. Following transfer of CFSE-loaded, unfractionated donor splenocytes, we identified proliferating donor T cells by flow cytometry as Ki-67⁺, CFSE^{dim} (at least one division). By day 3 after transfer, B10.D2 CD4 T cells exhibited a significantly greater percentage of dividing CD4 and CD8 T cells compared with DBA donors (Fig. 3A, 3B). By day 4, the percentage of dividing DBA T cells had increased for both CD4 and CD8 T cells, but CD4 values were still significantly reduced for DBA versus B10.D2 donors. A reciprocal analysis of undivided cells (Ki-67[−], CFSE^{bright}) confirmed these results and demonstrated that DBA→F1 mice have a significantly greater percentage of undivided CD4 T cells (Fig. 3C) and CD8 T cells (Fig. 3D) at day 3. These differences persist at day 4 for CD4 T cells but narrow for CD8 T cells. These results support a defect in the initial activation kinetics of both DBA CD4 and CD8 T cells versus B10.D2 mice. Lastly, a kinetic analysis of splenic cytokine gene expression from the combined cohorts in Figs. 1–3 demonstrates a significant burst of IFN- γ at day 7 for B10.D2→F1 mice that is not seen in DBA→F1 mice (Fig. 3E), replicating the results when comparing B6→F1 and DBA→F1 mice (20, 22). Thus, DBA→F1 mice exhibit impaired initial expansion

of both DBA CD4 and CD8 T cells, followed by impaired Th1 cytokine expression at days 5–7. The DBA CD8 expansion defect could be secondary to impaired DBA CD4 help, as suggested by the defective day-3 DBA CD4 proliferation (Fig. 3A); however, an intrinsic DBA CD8 defect cannot be excluded by these data.

Results following transfer of purified donor CD4 and CD8 T cells

DBA CD4 T cell help for CD8 CTLs is defective. CD4 T cell help is critical for the maturation of naive CD8 T cells into effector and memory CTLs (31). The mechanism by which CD8 CTL maturation is defective in DBA→F1 mice (22) involves at least an intrinsic defect in DBA CD8 T cells (32); however, the impaired proliferation of DBA CD4 T cells in Fig. 3 raises the possibility that a secondary defect in DBA CD4 T cell help for DBA CD8 CTL maturation may also be present. To address the individual roles of CD4 and CD8 T cells in generating in vivo CD8 effector CTL function, CD4 and CD8 T cells from B10.D2 and DBA donors were purified by negative isolation and then paired in a mixed or matched strain manner prior to transfer into BDF1 hosts. To better detect a CD4 helper defect for CD8 CTLs, donor inocula were equalized to contain an off-plateau dose of donor T cells at the lower limit of acute GVHD induction (7×10^6 CD4 and 4×10^6 CD8 T cells), as previously demonstrated (33, 34). Elimination of host B cells at days 10–14 was shown to be a sensitive indicator of in vivo donor CTL killing in this model (22). Comparing day-14 host B cell numbers, control matched B10.D2 CD4 + B10.D2 CD8 (matched B10.D2→F1) donor cells induced a significant and profound host B cell elimination compared with uninjected F1 mice (Fig. 4A, bars 1 and 2), typical of an acute GVHD cytotoxic phenotype and indicative of a strong B10.D2 CD8 CTL response (35). In contrast, matched DBA CD4

FIGURE 3. Initial DBA donor T cell proliferation is defective compared with B10.D2 T cells. BDF1 mice received CFSE-labeled DBA or B10.D2 (unfractionated) donor splenocytes and were assessed at days 3 and 4 in separate experiments by flow cytometry expression of Ki-67 and CFSE (see *Materials and Methods*). Proliferating donor CD4 (**A**) and CD8 (**B**) T cells are shown as the percentage of Ki-67⁺, CFSE^{dull} cells. Nonproliferating CD4 (**C**) and CD8 (**D**) cells are shown as the percentage of Ki-67⁻, CFSE^{bright} cells. Donor cells were examined by flow cytometry prior to transfer, and the inocula were adjusted so that each contained 5×10^6 CD8 T cells and $7.8\text{--}9.6 \times 10^6$ CD4 T cells. (**E**) Spleens from the cohorts in Figs. 1A–D and 3 also were examined by real-time PCR for IFN- γ expression as a function of time after donor cell injection. Data are group mean \pm SE ($n = 5$ mice/group). * $p < 0.05$, *** $p < 0.001$.



and DBA CD8 donor T cells (matched DBA→F1) induced a significant host B cell expansion typical of the expected day-14 stimulatory chronic GVHD (Fig. 4A, bars 1 and 3, respectively), consistent with a failure of DBA CD8 CTL maturation. These positive controls for acute and chronic GVHD phenotype dem-

onstrate that purification and recombination of CD4 and CD8 T cell subsets do not alter the expected 2-wk phenotypes observed in unfractionated donor splenocyte transfer experiments (35) and that T cells are necessary and sufficient for GVHD induction.

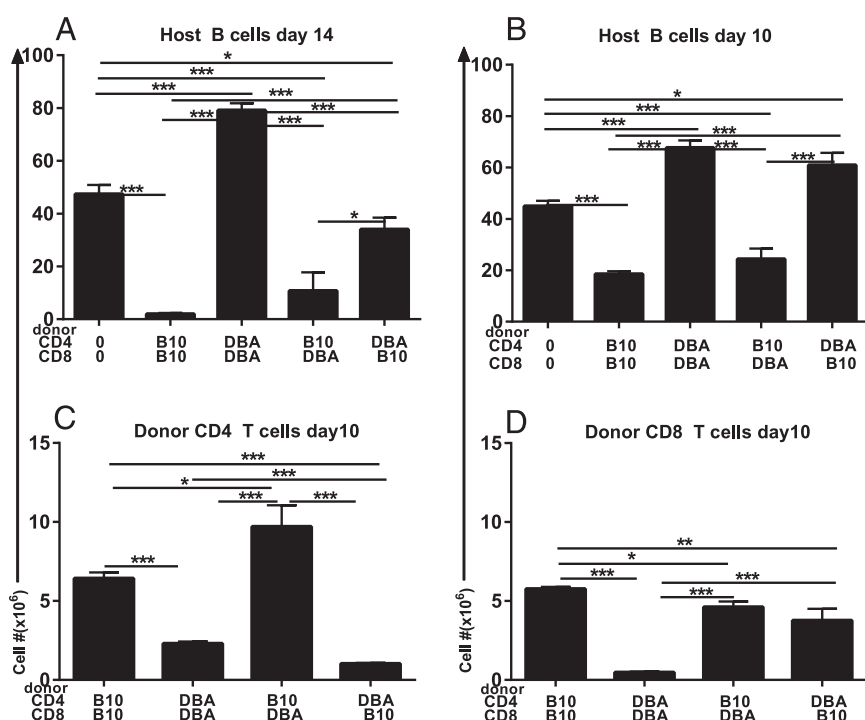


FIGURE 4. Defective DBA CD8 CTLs reflect both an intrinsic CD8 defect and an extrinsic CD4 helper defect. CD4 and CD8 T cells were purified by negative isolation from DBA and B10.D2 spleens, as described in *Materials and Methods*. BDF1 mice received either no donor cells or 7×10^6 CD4 T cells and 4×10^6 CD8 T cells from donor strains, as designated on the x-axis. F1 mice were assessed by flow cytometry for host B cells at day 14 (**A**) and at day 10 (**B**), as well as for engraftment of donor CD4 (**C**) and CD8 (**D**) T cells at day 10. Data are group mean \pm SE ($n = 3\text{--}5$ mice/group). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Mixing of donor T cell subsets further demonstrates the strain-specific contribution for each subset. Strain differences in the strength of CD4 T cell help are seen by keeping the CD8 T cell strain constant and varying the source of CD4 T cells. For B10.D2 CD8 T cells, significant and profound killing of host B cells (versus control F1) is seen when paired with B10.D2 CD4 T cells (Fig. 4A, bars 1 and 2); however, killing is significantly reduced when paired with DBA CD4 T cells (Fig. 4A, bar 5 versus bar 2), indicative of a severe defect in DBA CD4 T cell help. Conversely, DBA CD8 T cell killing of B cells is profoundly impaired (versus control F1) when paired with DBA CD4 T cells, but it is significantly boosted to levels comparable to matched B10.D2→F1 mice (Fig. 4A, bar 2) by pairing with B10.D2 CD4 T cells (Fig. 4A, bars 1, 3, and 4). Thus, regardless of the strain of CD8 T cell, CD4 T cell help is significantly reduced for DBA mice compared with B10.D2 mice.

Conversely, an intrinsic CD8 T cell defect can be assessed by keeping the strain of CD4 T cell help constant and varying the strain of CD8 T cells. Using this approach, B10.D2 CD4 T cells promote profound and statistically comparable (p = not significant) CD8 CTL elimination of host B cells (versus control F1), regardless of the strain of the donor CD8 T cell (Fig. 4A, bars 1, 2, and 4). A different effect is seen with DBA CD4 T cells. The impaired host B cell killing observed when DBA CD4 T cells are paired with DBA CD8 T cells is significantly boosted (but not normalized) by pairing of DBA CD4 T cells with B10.D2 CD8 T cells (Fig. 4A, bars 1, 3, and 5). The difference in killing (i.e., B cell elimination) seen for mixed DBA CD4 + B10.D2 CD8→F1 mice versus matched DBA→F1 mice (Fig. 4A, bar 5 versus bar 3) reflects an intrinsic DBA CD8 T cell defect that can also be significantly corrected with fully functional B10.D2 CD4 help (Fig. 4A, bar 4) but is more pronounced when CD4 help is impaired (Fig. 4A, bar 3).

Using this same approach of mixing or matching donor T cell subsets, a similar effect was seen at day 10, prior to maximal B cell elimination seen on day 14 (Fig. 4B). Keeping constant the strain of CD8 T cells, B10.D2 CD8 T cells induced significant host B cell killing when paired with B10.D2 CD4 T cells (Fig. 4B, bars 1 and 2); however, pairing with DBA CD4 T cells significantly abrogated B cell elimination to levels comparable to uninjected control F1 mice (Fig. 4B, bars 1, 2, and 5). Similarly, DBA CD8 T cells paired with B10.D2 CD4 T cells induced significant host B cell killing, which was comparable to matched B10.D2→F1 mice (Fig. 4B, bar 2 versus bar 4, p = not significant), compared with uninjected F1 mice (Fig. 4B, bar 4 versus bar 1). This strong DBA CD8 T cell killing was completely abrogated when paired with DBA CD4 T cells (Fig. 4B, bar 3 versus bar 4), confirming the CD4 helper defect demonstrated in Fig. 4A. Conversely, comparisons keeping constant the strain of CD4 T cells demonstrated that B10.D2 CD4 T cells induced efficient B cell killing whether paired with B10.D2 CD8 T cells or DBA CD8 T cells (Fig. 4B, bars 1, 2, and 4), whereas DBA CD4 T cells induced a statistically similar failure of CD8 CTL killing of B cells whether paired with DBA CD8 T cells or B10.D2 CD8 T cells (Fig. 4B, bars 1, 3, and 5), indicative of a significant DBA CD4 T cell helper defect. The intrinsic DBA CD8 defect at day 14 (Fig. 4A, bars 3 and 5) was not detectable at day 10 (Fig. 4B).

Thus, using host B cell elimination as a marker of in vivo donor CTL activity, DBA CD4 T cell help for normal B10.D2 CD8 T cells was defective relative to the helper function of B10.D2 CD4 T cells at days 10 and 14. In contrast, defective DBA CD8 CTL function seen when paired with DBA CD4 T cells was significantly corrected and nearly normalized by pairing with B10.D2 CD4 T cells. A milder intrinsic DBA CD8 defect was seen only at day

14 and could be significantly boosted by B10.D2 CD4 T cell help. Moreover, at days 10 and 14, mixed B10.D2 CD4 + DBA CD8→F1 mice exhibited B cell killing comparable to control matched B10.D2→F1 mice (Fig. 4A, 4B, bars 2 and 4) and B cell killing significantly greater than mixed DBA CD4 + B10.D2 CD8→F1 mice (Fig. 4A, 4B, bar 5), indicating that B10.D2 CD4 T cell help compensates for an intrinsic DBA CD8 defect. Importantly, DBA CD4 T cell help cannot induce optimal CD8 CTL function in normal B10.D2 CD8 T cells (Fig. 4A, 4B, bar 2 versus bar 5). Together, these results strongly support a model in which defective DBA CTL generation is the result of a profound defect in DBA CD4 T cell help in combination with a milder intrinsic DBA CD8 T cell defect.

CD4 T cell help for CD8 engraftment and maturation is defective in DBA mice. In the p→F1 model of acute GVHD, donor CD8 T cell engraftment peaks at approximately day 10 and then undergoes homeostatic contraction (22). To further clarify the respective roles of DBA CD4 and CD8 T cells in defective CTL effector function, we examined peak (day 10) donor CD4 and CD8 engraftment in the cohort shown in Fig. 4B using mixed or matched donor T cell subsets. Despite injecting equal numbers of purified donor CD4 T cells, B10.D2 donors exhibited significantly greater CD4 engraftment compared with DBA donors, regardless of their CD8 T cell pairing. Specifically, B10.D2 CD4 engraftment (Fig. 4C, bars 1 and 3) was significantly greater than DBA CD4 engraftment (Fig. 4C, bar 2 or 4), consistent with their greater B10.D2 CD4 proliferation and expansion shown in Figs. 1–3.

With regard to CD8 T cell engraftment (Fig. 4D), control matched B10.D2→F1 mice exhibited the expected strong engraftment of CD8 T cells, whereas control matched DBA→F1 mice exhibited the expected significantly impaired CD8 engraftment (Fig. 4D, bars 1 versus bar 2), consistent with previous work (22). For experimental mixed groups, the defective DBA CD8 engraftment seen when DBA CD8 T cells are paired with DBA CD4 T cells (Fig. 4D, bar 2) was boosted significantly by pairing with B10.D2 CD4 T cells (Fig. 4D, bar 3), consistent with defective DBA CD4 help. However, the level remained slightly, but significantly, less than that of matched control B10.D2→F1 mice (Fig. 4D, bar 1), consistent with an intrinsic DBA CD8 defect. Similarly, the robust B10.D2 CD8 engraftment seen when paired with B10.D2 CD4 T cells (Fig. 4D, bar 1) was significantly reduced, but not abrogated, when B10.D2 CD8 T cells were paired with DBA CD4 T cells (Fig. 4D, bar 4), consistent with a DBA CD4 helper defect. Importantly, B10.D2 CD8 engraftment in DBA CD4 + B10.D2 CD8→F1 mice (Fig. 4D, bar 4) was significantly greater than that of DBA CD8 engraftment in matched control DBA→F1 mice (Fig. 4D, bar 2), indicative of an intrinsic DBA CD8 T cell defect, because both groups have the same source of CD4 T cell help.

Engrafted donor CD8 T cells were examined further for markers of effector maturation and activation to include Ki-67, SLECs (KLRG-1⁺, CCR7⁻), and intracellular IFN- γ , TNF, pfp, and GrB. Representative flow cytometry tracings are shown in Supplemental Fig. 2. All markers were readily detectable in matched B10.D2→F1 acute GVHD controls and were profoundly defective in matched DBA→F1 chronic GVHD controls, as expected (Fig. 5, bars 1 and 2, all panels). Experimental groups (Fig. 5, bars 3 and 4, all panels) receiving mixed donor strain T cells were largely intermediate between the two controls.

Comparisons keeping the CD8 T cell strain constant and varying the CD4 T cell strain demonstrated that, for B10.D2 CD8 T cells, all markers were expressed at strong levels when paired

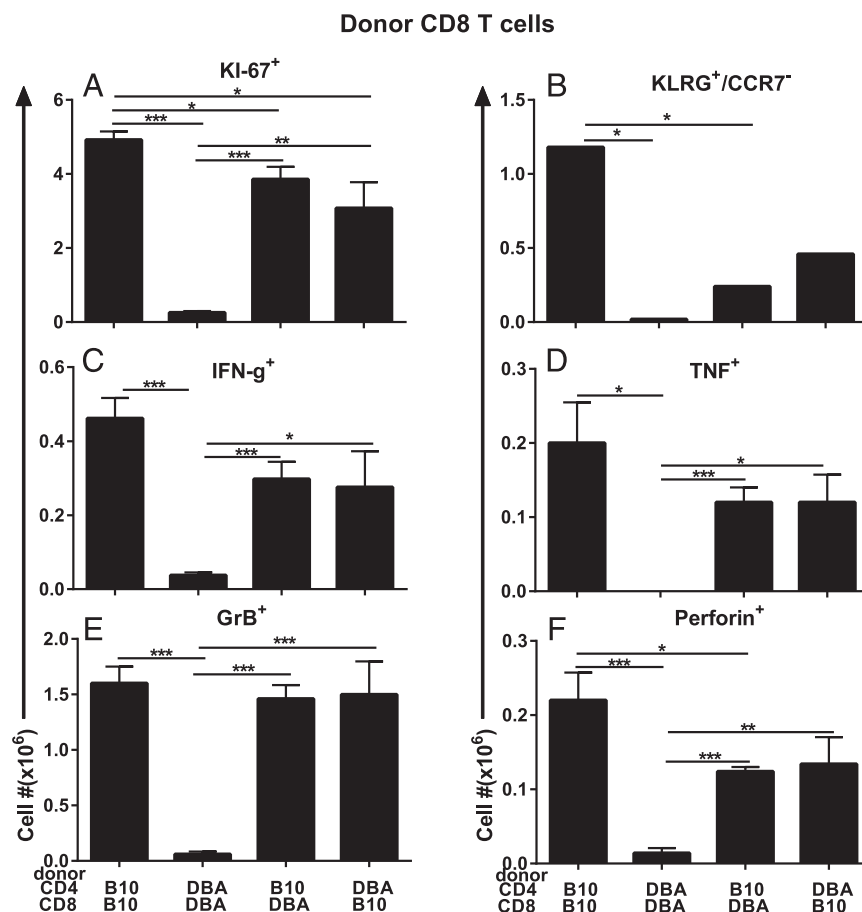


FIGURE 5. Intracellular markers of CD8 CTL effector maturation are defective in DBA CD8 T cells as the result of an intrinsic defect and an extrinsic DBA CD4 helper defect. The cohort shown in Fig. 4B–D was examined by flow cytometry at day 10 for donor CD8 T cell Ki-67⁺ proliferating cells (**A**) and SLECs (KLRG-1⁺, CCR7⁻) (**B**), as well as for intracellular expression of IFN-γ (**C**), TNF (**D**), GrB (**E**), and pfp (**F**). Data are group mean \pm SE ($n = 5$ mice/group). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

with B10.D2 CD4 T cells (Fig. 5, bar 1, all panels); however, pairing with DBA CD4 T cells (Fig. 5, bar 4, all panels) diminished B10.D2 CD8 expression of all markers, with the exception of GrB, but the data reached significance only for Ki-67. Similarly DBA CD8 T cells paired with DBA CD4 T cells exhibited profoundly impaired expression of all markers compared with matched B10.D2→F1 controls (Fig. 5, bar 1 versus bar 2, all panels); however, pairing DBA CD8 with B10.D2 CD4 improved (but did not normalize) the DBA CD8 defect (Fig. 5, bar 2 versus bar 3, all panels) for all markers. Significance was reached for all but KLRG-1⁺ SLECs, demonstrating that the intrinsic defect in DBA CD8 T cells can be significantly improved when defective DBA CD4 T cell help is replaced by functionally normal B10.D2 CD4 T cells.

Comparisons keeping the CD4 T cell strain constant and varying the CD8 T cell strain demonstrated that B10.D2 CD4 T cells induced strong expression of all markers in B10.D2 CD8 T cells (Fig. 5, bar 1, all panels). However, pairing of B10.D2 CD4 T cells with DBA CD8 T cells (Fig. 5, bar 3, all panels) yielded intermediate to normal levels of analyzed markers, consistent with a DBA CD8 intrinsic defect. DBA CD4 T cells induced poor expression of all markers in DBA CD8 T cells (Fig. 5, bar 2, all panels), but pairing with B10.D2 CD8 T cells (Fig. 5, bar 4, all panels) resulted in significantly greater expression of all markers with the exception of KLRG-1⁺ SLECs, further supporting the conclusion that there is an intrinsic defect in DBA CD8 T cells.

Taken together, the results of Figs. 4 and 5 support the conclusion that DBA CD4 T cells are defective helpers for CD8 CTL maturation because defective DBA CD8 day-10 expansion/effector maturation and day-10–14 killing can be significantly corrected by B10.D2 CD4 T cell help, and DBA CD4 T cells are

unable to induce normal day-10 CTL parameters when paired with B10.D2 CD8 T cells. Conversely, evidence of an intrinsic DBA CD8 defect is seen by the failure of B10.D2 CD4 T cells to fully normalize DBA CD8 engraftment and effector marker expression.

Long-term effects: DBA CD4 T cells induce greater lupus-like renal disease regardless of CD8 T cell strain pairing. Using the same experimental protocol of mixing and matching donor T cell subsets described for Figs. 4 and 5, p→F1 mice were observed long term (14 wk) for evidence of T cell-driven B cell hyperactivity, as shown by serum anti-DNA Ab (Fig. 6A), and lupus-like renal disease, as shown by proteinuria (Fig. 6B). Control, matched DBA→F1 mice exhibited significant increases in anti-DNA Ab levels beginning at week 2 and peaking at week 12 compared with control uninjected mice, as well as proteinuria progressing to 3+ (Fig. 6A, 6B, ○), indicative of severe lupus-like nephritis and mimicking the severe lupus nephritis reported following the transfer of unfractionated DBA donor splenocytes into BDF1 mice (12). These results confirm that only T cells are required for disease (i.e., no donor APCs or non-T cells were injected); negative isolation of donor cells did not alter the expected phenotype long term. In contrast, no sustained significant elevations over uninjected control F1 mice were seen in either parameter for matched B10.D2→F1 mice (Fig. 6A, 6B, ●), consistent with previous reports of B6→F1 acute GVHD long-term survivors (36). For mice receiving mixed donor T cell subsets, sustained significant elevations in serum anti-DNA and in proteinuria were seen only in F1 mice receiving DBA CD4 T cells paired with B10.D2 CD8 T cells (Fig. 6A, 6B, □). Values did not differ significantly from control matched DBA→F1 mice. Conversely, F1 mice receiving mixed B10.D2 CD4 + DBA CD8 T cells (Fig. 6A, 6B, ■) exhibited an early significant increase in anti-DNA Ab; however,

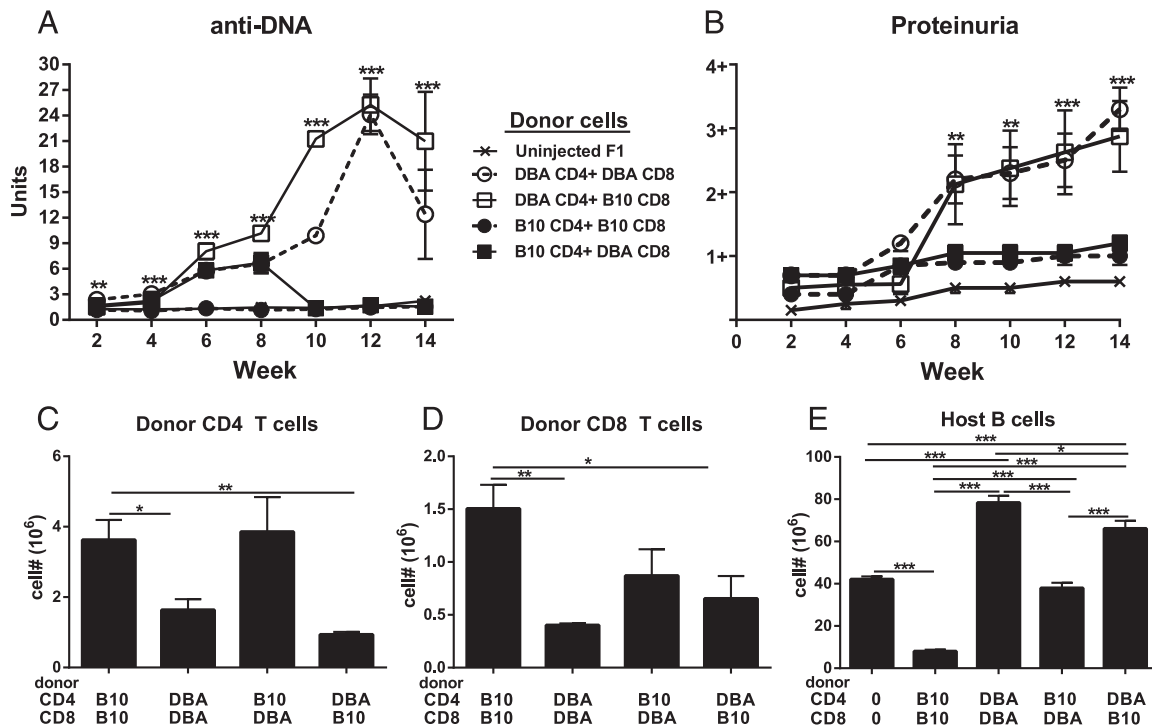


FIGURE 6. DBA CD4 T cells are lupus prone, regardless of the strain of paired CD8 T cells. Experimental protocol is as described in Fig. 4. F1 mice were assessed serially for serum anti-DNA Ab (**A**) and proteinuria (**B**). At 14 wk, F1 spleens were assessed by flow cytometry for donor CD4 T cells (**C**), donor CD8 T cells (**D**), and host B cells (**E**). Data are group mean \pm SE ($n = 3\text{--}5/\text{group}$). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

values returned to control levels by 8 wk. This transient increase in anti-DNA Ab levels was not accompanied by significantly elevated proteinuria. Renal histological analysis revealed lupus-like nephritis only in groups receiving DBA CD4 T cells (i.e., DBA CD4 + DBA CD8 \rightarrow F1 and DBA CD4 + B10.D2 CD8 \rightarrow F1; data not shown).

Long-term donor T cell engraftment results (Fig. 6C, 6D) mirrored the day 10 engraftment trends (Fig. 4C, 4D), with DBA CD4 T cell engraftment (regardless of CD8 pairing) being significantly lower than matched B10.D2 control CD4 T cells (Fig. 6C, bars 2 and 4 versus bar 1). Similarly, CD8 engraftment was significantly less for DBA matched controls versus B10.D2 matched controls (Fig. 6D, bars 1 and 2) and mixed groups exhibited impaired long-term CD8 engraftment versus matched B10.D2 \rightarrow F1 mice (Fig. 6D, bars 3 and 4 versus bar 1). Despite the reduced DBA CD4 engraftment, host B cell expansion was significantly greater in F1 mice receiving DBA CD4 T cells versus B10.D2 CD4 T cells, regardless of the CD8 T cell pairing (Fig. 6E, bars 3 and 5 versus bars 2 and 4). Taken together, the data in Fig. 6 support the conclusion that DBA CD4 T cells exhibit greater lupus-inducing potential than do B10.D2 CD4 T cells, despite their reduced long-term engraftment compared with B10.D2 CD4 T cells. Furthermore, the lupus-promoting effect of DBA CD4 T cells was not mitigated by the presence of intrinsically normal B10.D2 CD8 T cells, likely as a result of poor CD4 help for B10.D2 CD8 CTLs.

Results following transfer of purified CD4 donor T cells alone

Qualitative differences in donor CD4 activation: B10.D2 CD4 T cells promote CD8a⁺ DCs and CMI, whereas DBA CD4 T cells promote plasmacytoid DC expansion and severe lupus. In the p \rightarrow F1 model, transfer of parental CD4 T cells in the absence of CD8 T cells induced a lupus-like chronic GVHD; however, the degree of resemblance to human lupus and the severity of disease varied with the parent and the F1 strain combinations used (14). Although host

genetics were shown to contribute to disease expression (11), the role of donor CD4 T cells in shaping disease outcome has not been fully addressed. The foregoing data strongly support the idea that DBA CD4 T cells exhibit qualitative differences in vivo compared with B10.D2 CD4 T cells and that these qualitative differences result in a differential lupus-inducing proclivity. To address this possibility, we compared immunologic parameters in BDF1 mice at 2 and 12 wk after the transfer of purified DBA (DBA CD4 \rightarrow F1) or B10.D2 CD4 T cells (B10.D2 CD4 \rightarrow F1). In the absence of parental strain CD8 T cells, host splenocytes were not eliminated in either transfer at days 10–14, and mice developed a chronic GVHD phenotype both short and long term (10, 33, 37).

Following transfer of equivalent numbers of donor CD4 T cells, DBA CD4 \rightarrow F1 mice exhibited significantly greater donor CD4 engraftment compared with B10.D2 CD4 \rightarrow F1 mice at weeks 2 and 16 (Fig. 7A). These results are in contrast to Figs. 3 and 6, where B10.D2 CD4 T cells are significantly greater than DBA CD4 T cells both short and long term, a difference that likely relates to the coinjection of donor CD8 T cells, which were reported to promote CD4 expansion (30). Greater DBA CD4 engraftment is associated with significantly greater DBA CD4 Tfh CD4 cells short and long term (Fig. 7B). Although these levels of CD4 Tfh cells are very low for DBA donors, they are detectable, whereas values for B10.D2 donors are below the limits of detection. With regard to host CD4 T cells, DBA CD4 \rightarrow F1 mice also exhibited a significant increase over control F1 mice in the numbers of host total CD4 and Tfh CD4 cells both short and long term (Fig. 7C, 7D). No significant increase over control F1 mice for either of these host parameters was seen for B10.D2 CD4 \rightarrow F1 mice at either time. An increase in host CD4 T cell numbers is typical of chronic GVHD (12), and these results support the idea that chronic GVHD is severe in DBA CD4 \rightarrow F1 mice and mild in B10.D2 CD4 \rightarrow F1 mice.

Total host B cell numbers (Fig. 7E) and GC B cells (GL-7⁺) (Fig. 7F) were significantly elevated over control for both B10.D2

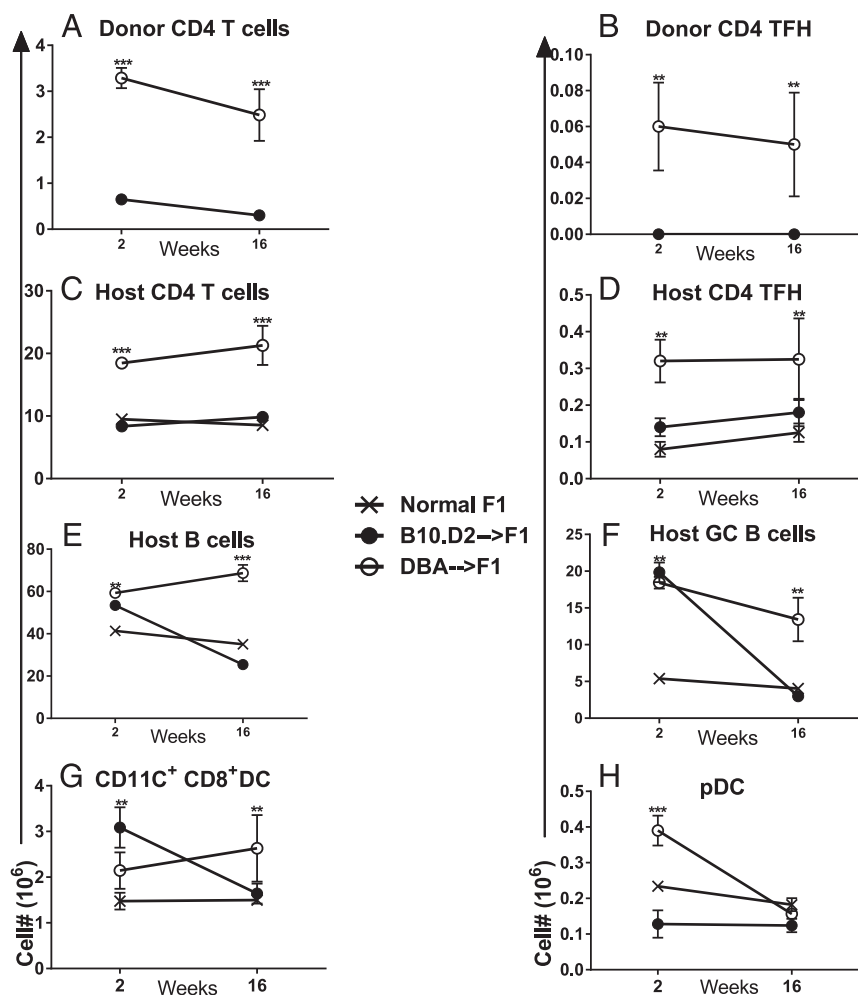


FIGURE 7. Purified DBA CD4 T cells induce greater lupus-like parameters than do purified B10.D2 CD4 T cells. F1 mice received 15×10^6 CD4 T cells negatively isolated from either DBA or B10.D2 donor spleens. In two separate experiments, mice were evaluated at either week 2 or 16 for donor CD4 T cells (total) (**A**), donor CD4 Tfh cells (CXCR5⁺, PD-1⁺) (**B**), host CD4 T cells (total) (**C**), host CD4 Tfh cells (**D**), host B cells (total) (**E**), host GC B cells (GL-7⁺) (**F**), host CD8a⁺, CD11c⁺ DCs (**G**), and host pDCs (**H**). Data are group mean \pm SE ($n = 3-5$ /group for week 2 and $n = 5$ /group for week 16). ** $p < 0.01$, *** $p < 0.001$.

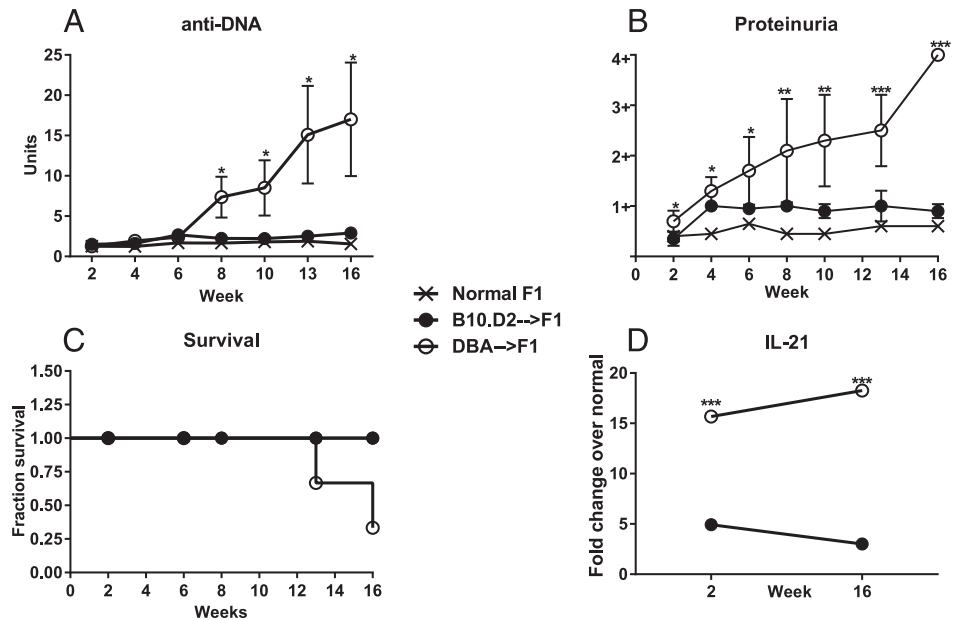
CD4 \rightarrow F1 and DBA CD4 \rightarrow F1 mice at 2 wk. However, by 16 wk, only DBA CD4 \rightarrow F1 mice continued to exhibit significant elevations over control mice with regard to either parameter. These data support the conclusion that only DBA CD4 \rightarrow F1 mice exhibit sustained CD4-driven B cell activation and autoantibody production. Lastly, striking differences in host APC subsets were observed. At 2 wk, B10.D2 CD4 \rightarrow F1 mice exhibited a significant (~ 3.5 -fold) increase in CD11c⁺, CD8a⁺ DCs compared with DBA CD4 \rightarrow F1 mice (Fig. 7G), whereas DBA CD4 \rightarrow F1 mice exhibited a significant (~ 2.5 -fold) increase in plasmacytoid DCs (pDCs) compared with B10.D2 CD4 \rightarrow F1 mice (Fig. 7H). Neither difference was maintained long term, and DBA CD4 \rightarrow F1 mice had a slight, but significant, increase in CD11c CD8a⁺ DCs compared with B10.D2 \rightarrow F1 mice at week 16. These results suggest that differential DC subpopulation expansion is an important early event in disease induction.

Taken together, the flow cytometry data in Fig. 7 indicate more severe and sustained CD4 T cell-driven, B cell activation for DBA CD4 \rightarrow F1 mice than for B10.D2 CD4 \rightarrow F1 mice. This conclusion is further supported by significantly greater serum anti-DNA Ab levels (Fig. 8A) and significantly greater proteinuria (Fig. 8B) in DBA CD4 \rightarrow F1 mice compared with B10.D2 CD4 \rightarrow F1 mice. The more severe renal disease was associated with earlier mortality in the DBA CD4 \rightarrow F1 group (Fig. 8C), prompting termination of the experiment and assessment of the surviving mice. Lastly, splenic IL-21 gene expression was significantly greater for DBA CD4 \rightarrow F1 mice compared with B10.D2 \rightarrow F1 mice at both 2 and 16 wk (Fig. 8D). Regarding gene expression of other cytokines, splenic

IL-4 expression for DBA CD4 \rightarrow F1 mice was significantly elevated at 2 wk compared with B10.D2 CD4 \rightarrow F1 mice; however, by 16 wk, the increases were no longer significant (data not shown). Conversely, IFN- γ levels were elevated at 2 wk for B10.D2 CD4 \rightarrow F1 mice compared with DBA CD4 \rightarrow F1 mice, but the difference failed to reach statistical significance (data not shown). Expression of other splenic cytokines (IL-6, IL-10, Mx-1, OAS) was increased ≤ 3 -fold compared with control F1 mice in both groups at both time points and was noninformative (data not shown).

Two distinct gene expression signatures in DBA CD4 \rightarrow F1 mice versus B10.D2 CD4 \rightarrow F1 mice. The foregoing results support the conclusion that DBA CD4 T cells exhibit an immune activation pathway in vivo that is significantly different from that of B10.D2 CD4 T cells, despite both p \rightarrow F1 combinations exhibiting host B cell expansion and a chronic GVHD phenotype at 2 wk. To determine whether these two pathways exhibit distinct gene expression signatures, spleens from B10.D2 CD4 \rightarrow F1, DBA CD4 \rightarrow F1, and uninjected control mice were profiled by genome-wide gene expression analysis at day 14, using Illumina BeadArray methodology (see *Materials and Methods*). The complete results are available at <http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?token=sjkggcvxsvvqx&acc=GSE71611> (accession number GSE71611). Differential expression analysis resulted in the identification of >300 candidate genes with >4 -fold expression ratio and p value < 0.05 between DBA CD4 \rightarrow F1 mice and B10.D2 \rightarrow F1 mice (Supplemental Fig. 3). Gene set enrichment analysis identified a significant subset of 24 high-confidence immune response-associated candidate genes that completely stratifies

FIGURE 8. Purified DBA CD4 T cells induce greater lupus-like disease than do purified B10.D2 CD4 T cells. The cohort described in Fig. 7 was serially evaluated for serum anti-DNA Ab (A), proteinuria (B), and survival (C). (D) Splenic IL-21 gene expression. Data are group mean \pm SE. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.



the two conditions (Fig. 9). Previously reported increases in IL-4 mRNA levels for DBA→F1 mice (20) and, as also noted above, for DBA CD4→F1 mice were confirmed in DBA CD4→F1 mice, whereas B10.D2→F1 mice exhibited an increase in IFN- γ typical of acute GVHD (Fig. 9, rows 4, 9, and 12) (20). Moreover, CXCR5 (Fig. 9, row 3) was greater in DBA CD4→F1 mice, consistent with greater CD4 Tfh activity and B cell help. An analysis of T cell activation- and expansion-related genes (Supplemental Fig. 4) indicated that B10.D2 CD4 T cells induced more IL-31Ra, BCL2L11, CD28, and TNFRSF9 (4-1BB), whereas DBA CD4→F1 mice exhibited more BH3 interacting domain death agonist and protein kinase C α . Furthermore, control F1, DBA→F1, and B10.D2→F1 gene expression signatures are significantly stratified after unbiased hierarchical clustering (Fig. 9). The 3-fold increase in IL-21 mRNA for DBA CD4→F1 mice versus B10.D2 CD4→V1 mice seen in Fig. 8D was not strong enough to be detected as significantly different by gene array. Because differences in IL-2 by protein or mRNA are best seen during days 1–3 (20, 38), we did not include IL-2-related genes in our analysis. Taken together, the disparate gene expression in these two p→F1 combinations further supports the conclusion that CD4 T cells from B6 and DBA mice induce two highly distinct lymphocyte-activation pathways.

DBA CD4 T cells exhibit defective in vitro IL-2 production and intracellular signaling. The foregoing results demonstrate two distinct lymphocyte-activation pathways in B10.D2 CD4→F1 mice compared with DBA CD4→F1 mice. These observations suggest the existence of pre-existing intrinsic differences in the default activation pathways used by B10.D2 or DBA CD4 T cells, possibly as the result of intrinsic differences in TCR-stimulated IL-2 production. IL-2 has critical roles in naive CD4 T cell proliferation, in CD4 help for CD8 CTLs, and in IFN- γ expression (31, 39–41), all of which are defective in DBA CD4 T cells (Figs. 1, 3–5). These results are consistent with previous work demonstrating a relative defect for in vivo and ex vivo IL-2 production in DBA→F1 mice compared with B6→F1 mice (20, 38). To address whether the in vivo functional defects in IL-2 reflect a pre-existing intrinsic defect in DBA T cells, we examined in vitro IL-2 parameters following maximal TCR stimulation. Because host alloantigen recognition is not a concern, and B6 and B10.D2 behave similarly (i.e., strong Th1/CMI response), we compared anti-CD3/anti-CD28-stimulated DBA and B6 splenocytes and

observed significantly impaired IL-2 protein and mRNA expression by DBA splenocytes (Fig. 10A, 10B).

To confirm the CD4 specificity of the IL-2 defect, purified CD4 T cells pooled from age- and sex-matched donors were tested in parallel for in vitro IL-2 parameters and intracellular signaling.

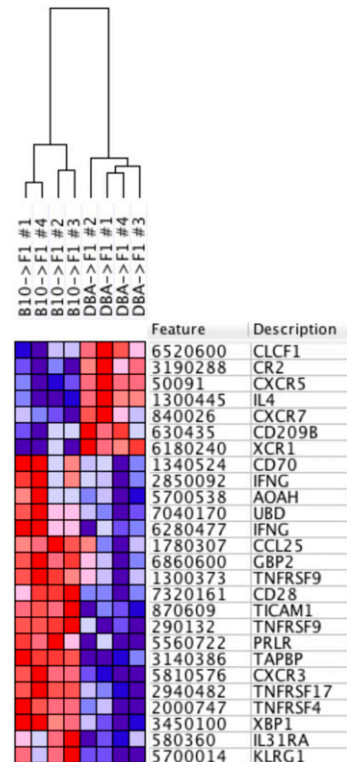
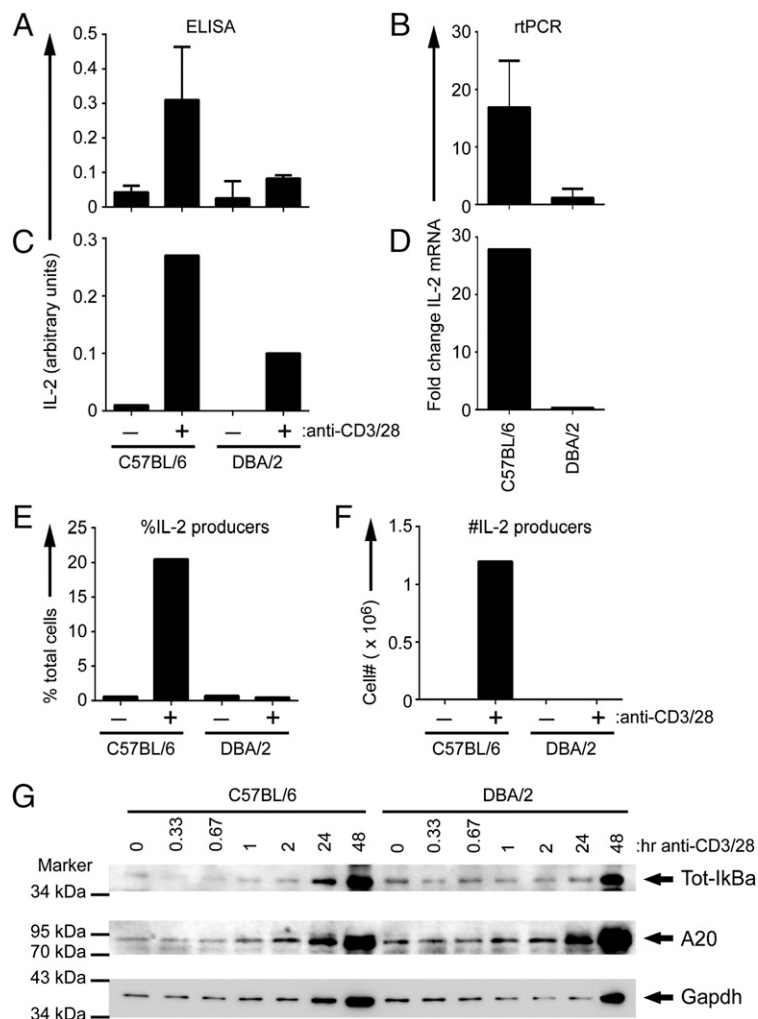


FIGURE 9. DBA CD4→F1 versus B10.D2→F1 mice exhibit two different pathways of immune gene activation. Purified donor CD4 T cells were transferred into BDF1 mice, as described for Figs. 7 and 8, and host spleens from B10.D2 CD4→F1 and DBA CD4→F1 mice ($n = 4$ /group) were profiled by whole-genome expression analysis at day 14 using Illumina BeadArray methodology (see *Materials and Methods*). Heat map of differentially expressed genes (red = higher, blue = lower expression) was generated by data output from GenomeStudio and analysis using GenePattern and Gene Set Enrichment Analysis.

FIGURE 10. Defective IL-2 production and intracellular IL-2 signaling in DBA CD4 T cells. B6 or DBA mice were tested for maximal IL-2 production using unfractionated splenocytes (**A** and **B**) or purified CD4 T cells (**C–F**), as described in *Materials and Methods*. At 48 h, supernatants were harvested and tested for IL-2 content by ELISA (**A** and **C**), and cells were harvested for IL-2 mRNA expression by PCR, which is shown as fold increase over unstimulated (**B** and **D**), or for intracellular IL-2 expression by flow cytometry, which are shown as the percentage of positive cells (**E**) or as the total number of cells (**F**). For all wells, the number of plated DBA CD4 T cells and B10.D2 CD4 T cells was equal. In preliminary studies, we were unable to detect IL-2 by ELISA before 48 h. Data in (**A**) and (**B**) represent group means \pm SE ($n = 4$ mice/group). For (**C**)–(**E**), spleens were pooled from 12 mice/group. (**G**) The same population of purified CD4 T cells used in (**C**)–(**F**) was stimulated for the indicated times with plate-bound anti-CD3 (145-2C11; 100 μ g/ml) and anti-CD28 (37.51; 10 μ g/ml). Whole-cell lysates were separated by SDS-PAGE and probed with anti-I κ B α (Cell Signaling Technology; 9246), anti-A20 (Santa Cruz Biotechnology; sc-166692), and anti-GAPDH (Santa Cruz Biotechnology; sc-32233).



Defective in vitro IL-2 production and mRNA expression were seen for DBA versus B6 pooled CD4 T cells (Fig. 10C, 10D). Intracellular IL-2 expression was also impaired in DBA CD4 T cells (Fig. 10E, 10F) but was not associated with greater numbers or percentages of Foxp3⁺ CD4 T cells (data not shown). Thus, DBA CD4 T cells have a defect in IL-2 production at the level of transcription of the IL-2 gene.

The pooled CD4 T cells from B6 or DBA mice shown in Fig. 10C–F were also examined for TCR-dependent activation of NF- κ B, a key regulator of IL-2 transcription. To assess the kinetics of TCR-dependent NF- κ B activation, we monitored degradation of I κ B α . Prior to TCR stimulation, NF- κ B is sequestered in an inactive state in the cytoplasm by I κ B α . TCR activation triggers the proteasomal degradation of I κ B α , leading to nuclear translocation of NF- κ B. Thus, I κ B α degradation is a measure of NF- κ B activation. Stimulation of purified B6 CD4 T cells with anti-CD3 and anti-CD28 caused degradation of I κ B α at 20 and 40 min poststimulation. CD4 DBA T cells had higher basal levels of I κ B α and showed a more transient degradation of I κ B α that was only apparent at 20 min post-TCR stimulation (Fig. 10G). Additionally, we examined the glycolytic gene, GAPDH, which is induced by the autocrine action of IL-2 (42). By 24 h poststimulation, B6 CD4 T cells exhibited increased levels of I κ B α and GAPDH protein (Fig. 10G), indicative of NF- κ B-dependent IL-2 production (I κ B α is resynthesized in an NF- κ B-dependent manner). In contrast, these changes in protein expression in DBA CD4 T cells were delayed until 48 h poststimulation, consistent with the observed defect in IL-2 production by DBA T cells. Finally,

Western blotting showed that CD4 DBA T cells have higher basal levels of A20, a negative regulator of NF- κ B activation (43, 44) linked to SLE (45) (Fig. 10G). Also, TCR induction of A20 was more robust in DBA T cells, particularly by 48 h poststimulation. It should be noted that these studies were performed on purified donor strain CD4 T cells at up to 48 h of in vitro stimulation, whereas the gene array studies in Fig. 9 were performed on F1 mice 14 d after donor cell transfer. Because of these differences, NF- κ B-related genes were not included in the gene array studies.

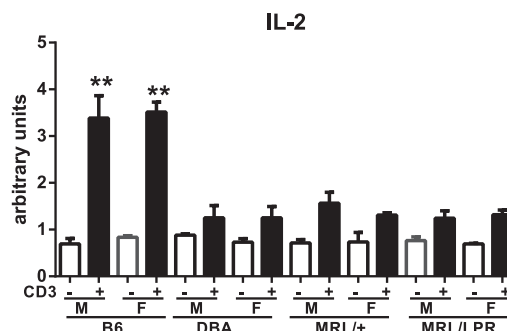


FIGURE 11. Defective in vitro DBA IL-2 production is comparable to that of MRL spontaneous lupus mice. Splenocytes from 10-wk-old male and female B6, DBA, MRL/+, and MRL/lpr mice were tested in vitro for anti-CD3 IL-2 production, as described in *Materials and Methods*. Data are mean \pm SE of three mice/group. Open bars represent unstimulated cells, and filled bars represent stimulated cells. ** $p < 0.01$.

Together, these data demonstrate that DBA CD4 T cells have a pre-existing defect in TCR activation of NF- κ B, which likely contributes to the observed impairment of IL-2 transcription. Increased basal and TCR-induced production of A20 may contribute to the poor NF- κ B response of DBA CD4 T cells.

Defective in vitro IL-2 production by DBA T cells is comparable to that of lupus-prone MRL mice. Defective in vitro IL-2 production has been well reported in both human and murine lupus (46). To determine the relative severity of the DBA IL-2 defect, we compared in vitro IL-2 production from young (10 wk) DBA splenocytes with that of young age-matched lupus-prone MRL/+ and MRL/lpr splenocytes. Both sexes were tested, and normal B6 splenocytes were the positive control. Confirming our results in Fig. 10A, DBA IL-2 production was significantly reduced compared with that of B6 mice but was not significantly different from MRL/lpr or MRL/+ mice (Fig. 11). Within a given strain, there were no significant sex differences in IL-2 production. These results indicate that the severity of the DBA IL-2 defect is comparable to that of young autoimmune-prone MRL mice.

Discussion

CD4 T cells play an essential role in lupus pathogenesis by providing help to autoreactive B cells that, in turn, produce the pathogenic autoantibodies mediating tissue injury (29, 47). This study advances our understanding of the critical role of CD4 T cells by demonstrating that pre-existing differences in the inherent strength of CD4 T cell help for B cells can shape the severity of lupus expression. Using MHC-identical but background gene-disparate strains, we observed that, following an experimentally induced loss of tolerance, CD4 T cells from H-2^d mice on the C57 black background induced transient autoantibodies without major lupus renal disease, whereas equivalent numbers of CD4 T cells from H-2^d DBA mice induced prolonged increases in autoantibody and severe lupus nephritis. These striking differences in the CD4 lupus-inducing potential were associated with two contrasting patterns of in vivo CD4 T cell activation. Naive B10.D2 CD4 T cells induced a strong Th1/CMI response that was characterized by a robust initial T cell proliferation, IL-2 and IFN- γ expression, and CD4 help for CD8 CTL effector maturation, with skewing toward CTL-promoting CD8a⁺ DC expansion and away from pDC expansion. This pattern was seen in association with reduced CD4 Tfh cells, transient autoantibody production, and mild lupus nephritis. In contrast, CD4 T cells from H-2^d DBA mice exhibited weak initial proliferation, reduced IL-2 and IFN- γ expression, and poor help for CD8 CTLs, with skewing away from CD8a⁺ DC expansion and toward pDC expansion. This pattern was associated with greater CD4 Tfh cell numbers, sustained autoantibody production, and severe lupus nephritis. These results are consistent with reports that Tfh cells are increased in lupus (48) and that strong T cell stimulation is needed for Th1 differentiation but not for Tfh cell differentiation (49). Differential immune skewing of donor T cells was not secondary to host NK cell function.

An analysis of activated genes from whole spleens confirmed that two gene-activation pathways are induced. Three hundred genes exhibited significant differences between the two p \rightarrow F1 groups, and 24 high-confidence immune-response gene differences were identified. B10.D2 CD4 \rightarrow F1 mice exhibited gene-expression changes that are typical of acute GVHD in B6 \rightarrow F1 mice or B10.D2 \rightarrow F1 mice (i.e., increased IFN- γ , KLRG-1, and TNFR-related genes with reduced IL-4). The reverse was seen in DBA CD4 \rightarrow F1 mice. The full implications of these differentially expressed genes require further study. However, these results support our conclusion that CD4 T cells exhibit strain-based dif-

ferences in their default helper response as a consequence of differential initial IL-2 production. The mechanism by which a defect in CD4 T cell IL-2 production skews toward excessive help for B cells and the potential role of regulatory T cells are under study.

These results also demonstrate that, following an experimental loss of tolerance, the inherent lupus-inducing proclivity of CD4 T cells is a reciprocal relationship between the ability to provide help for B cells and the ability to provide help for CD8 CTLs. Specifically, the striking lupus proclivity of DBA CD4 T cells relates to strong and sustained B cell help, coupled with impaired help for CD8 CTLs, whereas the weak lupus proclivity of C57/black background B10.D2 CD4 T cells relates to strong help for CD8 CTLs, coupled with reduced help for B cells, an effect that is seen even in the absence of donor CD8 T cells. The pattern of strong Th1/CMI and weak B cell help by B10.D2 CD4 T cells is also seen in B6 CD4 T cells, supporting a role for non-MHC genes (37). Because lupus-related autoantibodies are produced by host (F1) B cells, our results demonstrate that autoreactive B cells are normally present and quiescent in nonlupus-prone BDF1 mice; however, these B cells can be readily induced to secrete pathogenic autoantibodies following a loss of CD4 T cell tolerance, depending on the strength of CD4 T cell help for B cells.

Although an intrinsic CD8 defect in DBA CTL maturation was demonstrated (20, 32), its effect on CTL maturation appears to be modest compared with the more extensive extrinsic defect in DBA CD4 T cell help. The intrinsic DBA CD8 defect could be corrected largely, but not entirely, by normal B10.D2 CD4 T cell help, whereas the DBA CD4 helper defect strongly compromised the maturation of normal B10.D2 CD8 T cells. Importantly, pairing of DBA CD4 T cells with normal B10.D2 CD8 T cells did not mitigate the strong lupus-inducing proclivity of DBA CD4 T cells, indicating that, despite the important role played by CD8 T cells in downregulating lupus in the p \rightarrow F1 model and other murine models (see below), the lupus-downregulatory activity of CD8 T cells is critically dependent on CD4 T cell function.

The mechanisms by which CD4 T cells help CD8 T cells mature into effector CTLs were delineated recently (reviewed in Refs. 31, 39, 50–52). Briefly, following TCR engagement and CD28-mediated costimulation, CD8 T cells expand and express effector markers GrB, pfp, IFN- γ , and TNF. CD4 T cells help CTL maturation by licensing CD11c⁺, CD8a DCs through CD40–CD40L interactions and by providing IL-2, which drives both effector and memory CD8 development. Stimulation of CD8 T cells by licensed DCs in the absence of IL-2 induces effector CTL but poor memory CD8 function. IL-2 signaling through the high-affinity IL-2R directly induces T-bet expression, STAT-1 signaling, GrB/pfp upregulation, and effector expansion. These effects are directly proportional to the strength of IL-2 signaling. Inflammatory cytokines IL-12 and IFN- α , termed signal 3, also promote effector and memory CTL, in part by enhancing the sensitivity of CD8 T cells to IL-2 signaling. Thus, CD4 help for CD8 CTLs includes IL-2 production, licensing of CD11c⁺ DCs, and possibly other, undefined factors.

Our results demonstrating defective DBA CD4 T cell help for CTLs raised the possibility that DBA CD4 T cells have a pre-existing defect in IL-2 production. Previous work in the p \rightarrow F1 model using BDF1 mice as hosts was consistent with defective IL-2 production in DBA versus B6 donors. DBA \rightarrow F1 mice were reported to exhibit defective initial IL-2-dependent donor T cell proliferation, ex vivo IL-2 production, and IL-2 gene expression compared with B6 \rightarrow F1 mice (20, 38). Because IFN- γ production is largely IL-2 dependent (41), defective IFN- γ expression in DBA T cells is likely a consequence of impaired initial IL-2 production.

However, a direct comparison of IL-2 production by equal numbers of purified donor CD4 T cells was not performed in these studies. In the current study, equal numbers of donor CD4 T cells tested *in vivo* or *in vitro* revealed a striking defect in IL-2 production in DBA versus B10.D2 CD4 T cells.

Follow-up *in vitro* experiments were performed to show that DBA CD4 T cells have an intrinsic defect in TCR activation of NF- κ B, which may underlie the observed defect in IL-2 transcription. Mechanistically, the impaired activation of IL-2 in DBA CD4 T cells may be due to high basal and TCR-induced levels of A20, a ubiquitin-editing enzyme that negatively modulates cytoplasmic signaling to NF- κ B (43, 44). Importantly, polymorphisms in the human A20 gene region are associated with lupus (45). Therefore, our data suggest a possible mechanism connecting dysregulated expression of A20 in CD4 T cells with lupus. Thus, defective DBA CD4 help for CD8 CTL maturation is likely a combination of defective IL-2 production and defective licensing of CD8a⁺ DCs, as demonstrated in Figs. 7 and 10 and in a previous study (22). Conversely, pDCs were linked to lupus activity (53), and the excessive pDC activation seen in DBA→F1 mice (Fig. 7) may also inhibit CTL responses, because excessive pDC activation was reported to inhibit antiviral CTL responses (54).

The reduced CD8 CTL function seen in DBA→F1 lupus mice is a common feature in human and murine lupus. In general, CD8 T cells are critical in limiting tolerance breaks in normal mice, and they fail in lupus-prone mice (55). Defects in CD8-mediated lupus downregulation were reported in NZB/W (56–58), MRL/lpr (59, 60), and BxSB lupus mice (61, 62). Also, Qa-1–restricted CD8 T cells were reported to downregulate Qa-1–expressing Tfh cells and prevent lupus (63). In p→F1 mice, donor CD8 CTLs can prevent donor CD4-driven lupus (10, 64), and lupus develops when donor CD8 CTLs fail to mature (10). However, as our results demonstrate, the severity of the ensuing lupus in the absence of CD8 CTLs relates to the intrinsic lupus-inducing proclivity of the CD4 T cell.

In human lupus, CD8 CTL function is reported as defective both *in vitro* and *in vivo* (reviewed in Ref. 46). CD8 CTLs are absolutely required for control of EBV infection (65), and lupus patients exhibit impaired EBV CD8 CTL responses (66) and higher viral loads (67). As described above, IL-2 is important in CTL maturation, and defective IL-2 production is a longstanding and robust finding in both human and murine lupus (46, 68). Although APC defects contribute, lupus T cells exhibit an intrinsic defect in IL-2 production due to an imbalance in the CREM/CREB ratio in the IL-2 promoter region (69). Despite the robustness of defective IL-2 in lupus, its meaning remains incompletely understood. Specifically, does defective IL-2 production represent a primary, pre-existing defect central to lupus pathogenesis or is it a secondary defect that is just one of many examples of the disordered immunoregulation characteristic of lupus? The two possibilities are not mutually exclusive, and a secondary defect is well documented. In human lupus, defective *in vitro* IL-2 production varies with disease severity (70, 71). In spontaneous lupus mice, defective *in vitro* IL-2 production increases with age and disease severity (reviewed in Refs. 46, 68). In DBA→F1 mice, defective IL-2 production by normal BDF1 host splenocytes is observed as early as 2 wk after donor transfer, supporting the idea that defective IL-2 production can be induced and is an early marker of lupus activity (32). Lastly, imbalances in the CREM/CREB ratio in the IL-2 promoter region can be induced by lupus serum (69).

A primary, pre-existing defect in lupus T cell IL-2 production has been difficult to assess. The exact time of disease onset is unknown in spontaneous lupus mice, and prospective identification

of human lupus patients predisease is problematic. Additionally, the presence of a secondary IL-2 defect complicates detection of a primary defect. Nevertheless, supportive evidence is provided by Stohl et al. (72–74) who, using a redirected lysis assay and PBLs from monozygotic twins discordant for SLE, demonstrated defective killing by both the affected twin and the unaffected twin. These results are consistent with a primary lupus cytolytic defect that might be IL-2 dependent. A primary pre-existing defect T cell in IL-2 is further supported by our data demonstrating that DBA CD4 T cells exhibit defective *in vitro* and *in vivo* IL-2 production that, following an experimental loss of tolerance, results in immune skewing away from help for downregulatory CD8 CTLs and toward excessive help for B cells and sustained lupus.

It is important to note that DBA mice do not develop lupus spontaneously, despite exhibiting an IL-2 defect comparable to that of lupus-prone MRL mice. Thus, the primary IL-2 defect in DBA CD4 T cells by itself is not sufficient for lupus expression; however, following an experimental loss of tolerance (i.e., the transfer of DBA CD4 T cells into nonlupus-prone BDF1 hosts), florid lupus ensues in F1 mice. In contrast, lupus seen following the transfer of high IL-2–producing donor CD4 T cells (B10.D2 CD4→F1 or B6 CD4→F1) is milder, and tolerance is eventually restored. These strain-dependent differences in CD4 activation pathways demonstrate that the same initiating Ag (allogeneic host MHC II, I-A^b) can induce either a CMI/CTL response or a lupus response, depending on the pre-existing intrinsic response pattern of the responding donor CD4 T cells. Taken together, these results advance a novel paradigm in lupus pathogenesis. Currently, lupus is thought to result when a genetically predisposed individual encounters an environmental lupus trigger. Our results provide a mechanism by which environmental stimuli could induce lupus in a select group of individuals whose CD4 T cells exhibit a pre-existing DBA-like skewing toward prolonged help for B cells and away from IL-2 and help for CD8 CTLs. Should such lupus-prone individuals encounter a trigger requiring a strong CD8 CTL response for protective immunity (e.g., EBV), they exhibit skewing toward a nonprotective Ab response, resulting in persistence of Ag and immune stimulation rather than a protective CTL response with Ag clearance. Moreover, EBV is able to infect B cells in a non-Ag-specific manner through its binding to CD21, thereby rendering potentially all B cells, regardless of their Ag specificity, as “foreign” and potential targets for help by an oligoclonal population of Ag-specific CD4 T cells. This situation is analogous to DBA→F1 mice, in which an oligoclonal population of donor CD4 T cells provides help to all host MHC class II–disparate B cells, resulting in lupus-specific Ab, as previously described (10, 64). The link between EBV and human lupus is controversial; we are not proposing it is a causative trigger but rather that it exemplifies the qualities that a lupus trigger would require to induce disease in individuals with DBA-like CD4 T cell skewing (i.e., the ability to render potentially all B cells as foreign). Regardless of EBV’s etiologic role in lupus, the impaired CD8 CTL control of EBV in lupus patients discussed above is not necessarily indicative of EBV’s etiologic role, rather it is a manifestation of a lupus-related IL-2–mediated CTL defect, most likely secondary to disease but, in some instances, possibly due to primary DBA-like CD4 T immune skewing with impaired IL-2 production.

Clearly, lupus expression is a final common pathway of a number of immune defects. Because DBA→F1 mice strongly mimic the human lupus subset of renal disease, lupus-specific autoantibodies, reduced IL-2, and defective CD8 CTLs, the immune skewing shown by DBA CD4 T cells likely illustrates an important mechanism that is applicable to at least a subset of human lupus. Specifically, humans may exhibit genetically predetermined

DBA-like CD4 T cell skewing, which may itself be multifactorial to include individuals with a reduced intrinsic CD4 T cell IL-2 capacity. Such pre-existing, genetically determined nonadaptive immunologic skewing was documented in murine *Leishmania major* infection where B6 mice make a protective CMI response, and BALB/c mice make a nonprotective Ab response (75, 76). Moreover, experimental CMI promotion with rIL-12 in BALB/c mice was curative, whereas Ab promotion in B6 mice exacerbated disease (77). Similarly, immunomodulation in DBA→F1 mice also was effective, with agents that either enhance CTL formation or inhibit their downregulation preventing lupus short term (22, 34, 78). These results raise the possibility of a novel therapeutic approach in humans. Standard lupus therapy is associated with global immunosuppression. A variety of immune molecules is being targeted for blockade; however, these molecules will likely be accompanied by some level of immunodeficiency because targeted molecules are important in normal immune function. Our results support a new approach aimed at skewing CD4 T cells away from B cell help and toward help for CD8 CTLs (i.e., promoting CMI at the expense of Ab responses). Such an approach could be beneficial and would avoid the problems inherent in long-term immunosuppression.

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Disclosures

The authors have no financial conflicts of interest.

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