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Therapeutic potential of CD8+ cytotoxic T lymphocytes in SLE★

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Abstract

Recent evidence supports the idea that following a break in tolerance, CD8 cytotoxic T lymphocytes (CTL) may be an important but unrecognized mechanism for limiting expansion of autoreactive B cells. Failure of this mechanism could allow persistence of CD4 T cell driven polyclonal B cell activation resulting in clinical lupus. Although CD8 CTL failure may occur early in disease, work in mice supports the concept that therapeutic CTL enhancement may be both practical and beneficial in lupus. Devising such therapy for humans will first require an understanding of the in vivo mechanisms critical in CTL expansion and down regulation, particularly in the lupus setting which may differ from CTL generation in other clinical settings (e.g. tumors, infections).

Keywords

Lupus; Cytotoxic T cells; Graft-vs-host disease

SLE is an immune mediated, multi-system disease characterized by pathogenic autoantibodies against nuclear antigens [1]. T cells, particularly CD4 T cells are necessary and sufficient for lupus induction and are central in driving B cell production of autoantibodies in both human and murine lupus (reviewed in [2]). Although a large number of immune abnormalities have been reported in lupus [2], it is not clear which defects are primary and thereby predispose to disease and which defects are secondary to the altered immunoregulation characteristic of lupus. A major unresolved question in lupus pathogenesis remains the mechanism(s) by which T cell tolerance is lost and nuclear autoantigens targeted.

1. Downregulation in lupus

Experimentally induced tolerance breaks in normal mice do not invariably lead to autoimmunity. Instead, mice exhibit transient and self-limited autoantibody production without lupus-like organ disease [3–6]. Although the experimental conditions used in mice likely do not mimic the conditions that induce a loss of tolerance and lupus in humans, these results support that idea that endogenous downregulatory mechanisms exist that may prevent disease amplification and disease expression in normals and possibly fail in the lupus prone individual and allow progression to disease. Studies in both human and murine lupus indicate that CD8+ cytotoxic T lymphocytes (CTL) may be one of possibly several mechanisms that act to limit the development of lupus following a break in tolerance.

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2. CD8+ CTL downregulation in lupus

2.1. Human studies

The existence of a primary predisposing defect in CD8 CTL has long been suspected in human lupus but has been difficult to demonstrate because the lupus-inducing ag(s) is not known and may differ among patients, thereby preventing direct assessment of lupus-ag specific CD8+ CTL function. Additionally, active lupus in both humans and mice is associated with a secondary, non-specific defect in in vitro CTL responses to non-lupus ag(s) (reviewed in [7] further complicating the search for a primary defect. Despite these difficulties, supportive evidence has been provided by Stohl et al. [8–10] who, using a re-directed lysis assay and peripheral blood leukocytes from monozygotic twins discordant for SLE, demonstrated defective killing by both the affected and the unaffected twin. Although it was not possible to test CTL specificities against either self-ag or a putative lupus ag, these results support the existence in lupus patients of a primary cytolytic defect encompassing a variety of specificities possibly including the relevant lupus ags.

2.2. Murine studies

Studies in spontaneous murine lupus also support an important role for cytolytic mechanisms in delaying lupus expression. Both Fas and perforin pathways are important not only in normal lymphocyte homeostasis and downregulation of foreign ag-driven responses [11,12] but also in retarding lupus expression in MRL/+ mice. Fas-defective MRL/lpr mice exhibit lymphoaccumulation and accelerated lupus-like disease due to impaired Fas-mediated deletion of pathogenic T and B cells by Fas ligand (FasL) bearing CD4 and CD8 T cells [13]. Similarly, perforin gene inactivation in Fas-intact MRL mice (MRL/pfp KO) accelerates humoral autoimmunity and lupus-like disease compared to perforin-intact, Fas-intact MRL/+ mice [14] consistent with a major role for perforin, separate from Fas, in controlling B cell accumulation and autoimmunity in MRL/+ spontaneous lupus. Lastly, combined perforin-deficient, Fas-deficient MRL/lpr/pfp KO mice exhibit greater accumulation of double negative T cells and accelerated mortality compared with perforin-intact MRL/lpr mice indicating that perforin also downregulates autoreactive T and B cells independent of Fas. Importantly, defects in Fas or perforin do not cause lupus, as these studies show, but in the setting of lupus (e.g. MRL/+ mice), defects in one or both of these pathways accelerates disease. Thus, the two major CD8 CTL killing pathways (Fas and perforin) have a prominent role in down-regulation autoimmunity and retarding lupus expression.

3. Lupus development in the parent-into-F1 (P→F1) model of lupus-like graft-vs-host disease (GVHD)

A useful model for studying lupus is the P→F1 model of chronic GVHD (cGVHD) (reviewed in [15] in which normal homozygous parental T cells are transferred into normal non-autoimmune prone, unirradiated F1 mice. For example, the transfer of C57Bl/6 (B6) T cells (H-2^b) into B6D2F1 mice (BDF1, H-2^{b/d}), results in activation of allospecific donor T cells that recognize H-2^d of the opposite parent present on host cells. Although the recipient F1 is tolerant to donor H-2^b, host-vs-graft responses nevertheless do occur [16] but are overcome by the stronger donor anti-host response. Lupus-like cGVHD can be induced in BDF1 mice when MHC II disparate, parental strain B6 CD4 T cells are transferred. Because MHC II is expressed only on B cells and APC, the allospecific MHC II-restricted donor CD4 T cells provide cognate help (signal 2) to all host B cells resulting in polyclonal B cell expansion. Activated B cells that also encounter their BCR cognate ligand (signal 1) mature and secrete IgG. In the GVHD model, no foreign ag is administered, therefore only self-ag reactive B cells will encounter endogenous signal 1 and become autoantibody secreting

cells. IgG anti-ssDNA ab appear early (days 10–14) [17] and after this initial B cell expansion a second ag-driven phase ensues at >1 month of disease [15] characterized by detection of lupus-specific auto-antibodies not seen earlier e.g., anti-dsDNA, Sm, chromatin [18,19] and poly(ADP-ribose) polymerase-1 (PARP-1) depending on the P→F1 combinations used [20]. Clinical renal disease becomes demonstrable at approximately 2 months. The phenotype in cGVHD mice is highly similar to human lupus and consists of: 1) lupus-specific autoantibodies, 2) lupus-like renal disease, 3) lupus-like Ig and C' deposition in the skin, 4) positive Coombs test and 5) a female predilection in DBA→F1 mice [15,21–23]. As with human lupus, organ specific autoantibodies are not observed in cGVHD mice [22].

4. Lupus is aborted in P→F1 GVHD mice by co-transfer of donor CD8+ CTL

The transfer of both CD4 and CD8 parental T cells into MHC I+ II disparate F1 mice initially results in donor CD4 driven polyclonal B cell activation as in cGVHD, however activation of donor CD8 T cells specific for allogeneic (host) MHC I results in anti-host CTL that eliminate host B cells (and T cells) preventing autoantibody production and lupus [24]. Long term, mice exhibit a disease similar to acute GVHD (aGVHD) in human bone marrow transplant recipients [25]. In aGVHD mice, near complete elimination of splenic B cells is seen at two weeks, well before the second ag-driven phase in which lupus-specific autoantibodies arise in cGVHD. By targeting allogeneic MHC I, all B cells (autoreactive and foreign-reactive) are eliminated, thereby removing the potential for lupus development.

5. Surrogate markers for long term clinical GVHD phenotype

Previous work has demonstrated that as early as two weeks after donor cell transfer, recipient F1 mice exhibit what we have termed either a “stimulatory” or “cytotoxic” phenotype that can serve as a surrogate marker for the long term development of either lupus-like or acute GVHD-like clinical disease phenotype respectively [25,26]. The key features of each phenotype are shown in Table 1. Specifically, the transfer of both CD4 and CD8 B6 parental T cells into BDF1 mice results in aGVHD and clinical phenotype consisting primarily of mortality at >2 weeks of disease. At 14 days after donor cell transfer, mice exhibit a cytotoxic phenotype characterized by engraftment of both donor CD4 and CD8 T cells, anti-host CTL activity, near complete elimination of host B cells and strong upregulation of IFN- γ dependent Fas and FasL. In contrast, the transfer of B6 donor T cells containing only the CD4 subset into BDF1 mice results in lupus long term and a stimulatory two week phenotype characterized by engraftment of donor CD4 T cells only (no donor CD8 engraftment), no donor anti-host CTL activity, B cell expansion, autoantibody production and minimal upregulation of Fas and FasL. Surprisingly, transfer of both CD4 and CD8 T cells from the opposite parent, DBA/2 (DBA) into BDF1 mice, results in a stimulatory phenotype at two weeks rather than the expected cytotoxic phenotype due to defective DBA CD8 T cell maturation into CTL [24]. The two week stimulatory phenotype in DBA→F1 mice is associated with lupus-like renal disease long term. Importantly, because CD8 T cells are transferred in the donor inoculum, the DBA→F1 model is useful for identifying in vivo treatments that promote CTL activity and as a result convert the stimulatory phenotype to a cytotoxic phenotype [27,28]. Conversely, the strong CD8 CTL response in B6→F1 mice is useful for detecting in vivo treatments that selectively inhibit CD8 CTL (but not CD4 Th cells) and convert the cytotoxic phenotype to a stimulatory phenotype e.g., TNF- α blockade [29].

6. The impaired CTL phenotype is a new surrogate phenotype

Donor CD8 T cell engraftment and anti-host CTL activity determine whether P→F1 mice exhibit a cytotoxic phenotype (CTL function normal) or stimulatory phenotype (CTL

function absent) at two weeks of disease [24]. However our group has observed that a variety of intermediate impairments in CTL function result in intermediate phenotypes (Table 2), termed “impaired cytotoxic” phenotype. This phenotype can be further subdivided based on the number of residual host B cells at two weeks and designated as mild (B cells significantly<normal), moderate (B cells near-normal) or severe (B cells significantly>normal). The greater the number of host B cells present, the more severe CTL impairment. Interfering with CTL formation either by blocking CTL induction e.g., blockade of TNF- α [29] or IL-2 [30] or blocking effector function e.g. transferring donor cells deficient in perforin [31] or FasL [32] selectively impairs CTL elimination of B cells but does not impair donor CD4 T cell driven help for B cells and autoantibody production. For example, Fas/FasL and perforin are the two major pathways by which donor CD8 CTL eliminate host B cells [33]. The induction of acute GVHD using perforin-deficient (pfp KO) B6 donor cells results in a mildly impaired cytotoxic phenotype at two weeks due to the loss of one of the major CTL killing pathways. Despite the aGVHD like phenotype at two weeks, long term, pfp KO→F1 mice develop lupus [31]. This outcome reflects two processes: a) normal downregulation of CD8 CTL effectors at about two weeks [34] in the setting of incomplete elimination of host B cells; b) continued stimulation of donor CD4 T cells by residual host B cells resulting in persistent autoantibody production and progression to lupus.

These results in pfp KO→F1 mice indicate that failure of CTL to completely eliminate host B cells, in the setting of CD4 driven B cell activation is a final common pathway for the development of lupus in this model and raises the question as to whether a similar failure of CD8 CTL to eliminate all autoantibody secreting B cells allows continued CD4 driven B cell activation in humans allows the eventual development of lupus. This possibility will be difficult to confirm in human lupus as it will require testing of lupus-ag specific CD8 CTL function, preferably at the pre-clinical phase, neither of which is feasible at present.

7. Will promoting CD8 CTL in lupus patients be both beneficial and practical?

Regardless of the exact role CD8 CTL play in preventing lupus initiation and expression, it is possible that therapeutic enhancement of CD8 CTL may independently be beneficial in controlling established disease. Global B cell depletion has proven beneficial in lupus, however B cell depleting agents such as Rituximab can also cause hypogammaglobulinemia [35]. Clearly selective depletion of only the pathogenic autoantibody secreting B cells is preferable.

Ideally, potential therapeutic enhancement of endogenous CTL would target only pathogenic B cells. Recent work by Fan and Singh [36] supports the feasibility of this idea by identifying lupus-specific pathogenic peptides important in disease expression in the NZB/W model of spontaneous lupus. Vaccination with genes encoding those pathogenic peptides that are also recognized in conjunction with MHC I resulted in CTL that killed peptide-expressing, autoantibody producing B cells and improved disease. These results provide conceptual support for therapeutic CTL promotion in lupus by demonstrating: 1) the feasibility of generating appropriately targeted CTL in the setting of altered immunoregulation characteristic of lupus; and 2) that such CTL are beneficial.

8. How can protective CTL be induced if the ag is unknown?

Unlike NZB/W mice, the lupus-inducing ag in humans is not known and could differ between patients. The approach by Fan et al. [36] supports the rationale for promoting CTL as an adjunctive therapy in lupus, however a similar ag-specific approach in human lupus is

not currently feasible. An ag-independent approach however can be pursued based on the rationale that the relevant CTL precursors may likely be present in lupus patients and that defects in their activation/maturation can be bypassed by manipulating cytokines and/or costimulatory molecules. Devising such therapy for humans will first require an understanding of the in vivo mechanisms critical in CTL expansion and down regulation in the lupus setting which may differ from CTL generation in other clinical settings (e.g. tumors, infections). Animal models of lupus will be critical for generating the initial empirical data necessary for such an approach to proceed.

9. The DBA→F1 model is useful for identifying candidate approaches to in vivo CTL promotion that prevent lupus

Despite the transfer of donor CD8 T cells, DBA→F1 mice exhibit little demonstrable donor CTL effector function and consequently exhibit a stimulatory phenotype at two weeks and lupus-like renal disease long term [24,26]. Nevertheless, DBA donor CD8 T cells can be potentiated in vivo by rIL-12 administration thereby converting the stimulatory phenotype to a cytotoxic phenotype in DBA→F1 mice due to enhanced donor anti-host CTL [27]. We have observed similar results with selected CpG ODNs that induce IL-12 production [37] or by administration of an agonist CD40 mAb [38]. These agents were effective when administered during the first five days after donor cell transfer indicating that the common feature of these approaches is potentiation of CTL induction. An alternative approach is to block CTL downregulation and is demonstrated by the induction of an acute GVHD-like cytotoxic phenotype in DBA→F1 mice following blockade of CD80 mediated downregulation [28]. The conceptual validity of this approach has been further confirmed using Fas-deficient (B6 lpr) donor T cells and Fas-intact BDF1 recipients [39]. Lpr→F1 mice exhibit an impaired cytotoxic phenotype at two weeks due to an lpr-related CD4 helper defect. However, lpr CD8 CTL once generated exhibit defective downregulation due to defective Fas-mediated activation induced cell death. Donor CD8 CTL activity is potentiated and prolonged and as a result F1 mice do not develop lupus long term but instead develop acute GVHD.

Taken together, these results in DBA→F1 mice serve as proof of concept that ag-independent strategies for promoting CTL in vivo are possible and can be achieved by either promoting CTL maturation and effector development or by blocking down-regulation of effector CTL. Importantly, manipulations successful in promoting CTL and converting a stimulatory phenotype to a cytotoxic phenotype in the DBA→F1 GVHD model must be assessed long term for their ability to prevent the development of lupus. Moreover, these results support the possibility that it may be feasible to develop ag-independent strategies for promoting CTL that will improve human lupus. Prior to application to humans however, it will be important to confirm which of these approaches is both feasible and beneficial in other spontaneous murine lupus models.

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Take-home messages

- The parent-into-F1 (P→F1) model of graft-vs-host disease is an induced model of lupus that allows direct study of cytokines and costimulatory molecules important in T cell driven B cell hyperactivity or CTL generation.
- The two major clinical phenotypes in P→F1 mice are lupus-like disease seen in DBA→BDF1 mice and a lethal cytotoxic T cell mediated attack seen in B6→BDF1 mice.
- Both lupus-like disease and lethal disease exhibit surrogate markers at two weeks (stimulatory and cytotoxic phenotypes respectively).
- Agents that inhibit CTL in vivo are identified in B6→F1 mice by conversion of the cytotoxic to stimulatory phenotype.
- Agents that promote CTL in vivo are identified in DBA→F1 mice by conversion of the stimulatory to cytotoxic phenotype.
- The impaired cytotoxic phenotype is an intermediate phenotype and results from normal downregulation of CTL effectors prior to complete host B cell elimination.

Table 1

Two week phenotypes in P→F1 GVHD [26,40]

Parameter	Cytotoxic phenotype	Stimulatory phenotype
Donor T cells engrafted	CD4, CD8	CD4 only
Anti-host CTL	Present	Not detectable
Host B cells	<10% normal F1	150–200% normal F1
Serum IFN- γ	Strong elevation (≥ 100 -fold)	Mild elevation (≤ 10 -fold)
Fas/FasL upregulated	Strong	Minimal/mild
Autoantibodies	Transient	Sustained

Table 2

P→F1 combinations with cytotoxic, impaired cytotoxic or stimulatory phenotypes at 2 weeks

Donor→host	B cells (2 wk)	CTL impairment	Phenotype	Long term
B6 WT→BDF1 (control)	≤5–10% of normal	None	Cytotoxic	Acute GVHD
B6 pfp KO→BDF1	20–50% of normal	Mild (31)	Impaired Cytotoxic	Lupus ICGN
B6 lpr→BDF1	20–50% of normal	Mild (39)	Impaired Cytotoxic	Acute
B6 IFN- γ KO→BDF1	~normal F1	Moderate (40)	Impaired Cytotoxic	n.t.
B6 WT→BDF1+ anti-TNF	≥150% of normal	Severe (29)	Stimulatory	n.t.
B6 WT→BDF1+ anti-IL2	≥150% of normal	Severe (30)	Stimulatory	n.t.
B6 gld→BDF1	≥150% of normal	Severe (32)	Stimulatory	n.t.
DBA→BDF1 (control)	≥150% of normal	Severe (24)	Stimulatory	Lupus ICGN
DBA→BDF1 + rIL-12	≤5–10% of normal	None	Cytotoxic	n.t.
DBA→BDF1+ CpG	≤5–10% of normal	None	Cytotoxic	n.t.
DBA→BDF1+ antiCD40	≤5–10% of normal	None	Accelerated Cytotoxic	Lupus ICGN Blocked