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Defective *in vitro* IL-2 production in lupus is an early but secondary event paralleling disease activity: Evidence from the murine parent-into-F1 model supports staging of IL-2 defects in human lupus

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Abstract

T cell defects are a well described feature of both human and murine lupus however their exact significance is unclear. Evidence from an induced model of lupus, the P → F1 model of chronic lupus-like GVHD demonstrates that a secondary inducible T cell defect in *in vitro* IL-2 and CTL responses occurs early in the course of lupus-like disease and well in advance of clinical disease. Defective Th cell function was probed using a novel approach categorizing the response to two stimuli: 1) the MHC self restricted response, termed self + X; and 2) the allogeneic response. Using this approach, lupus mice exhibited similar *in vitro* Th cell pattern i.e. and absent S + X response but preserved allogeneic (termed -/+). In contrast, human lupus patients exhibited three possible response patterns, +/+, +/- or -/- with more severe *in vitro* T cell impairment correlated with more severe disease. Similarly, patients with other T cell mediated conditions i.e. HIV infection or renal allograft recipients, also exhibited more severe *in vitro* T cell impairment with greater disease activity or greater immunosuppression respectively. The similar Th response patterns in human and murine T cell mediated conditions indicates that the underlying mechanisms involved are not disease specific but instead reflect common immune responses and validate the use of the P → F1 model for future studies of T cell mediated conditions. These results support the use of prospective monitoring of IL-2 responses in lupus patients. Successful adaptation of this approach to the clinical setting could allow not only earlier therapeutic intervention and reduced organ damage but also earlier tapering of pharmacological agents and reduced untoward effects.

Keywords

Lupus; cytotoxic T cells; chronic graft-vs.-host disease; IL-2

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Defective cytotoxic T cell and IL-2 responses are frequent and well-established observations in both human and murine lupus

T lymphocyte abnormalities are a well-recognized accompaniment of systemic lupus erythematosus [1]. Lupus-related defects in T cell function comprise some of the earliest described and consistent observations, encompassing defects both *in vivo*, e.g. reduced delayed hypersensitivity [2,3], and *in vitro*, e.g. impaired antigen (ag)-driven T cell proliferation [4]. Lupus patients exhibit striking heterogeneity with respect to expression of both clinical disease and the presence of T cell defects. Nevertheless, defective cytotoxic T lymphocyte (CTL) responses are a longstanding observation [5–7] and perhaps the least disputed T cell abnormality in lupus [8]. Depressed *in vitro* effector CTL function likely reflects a more proximal defect in T cell production of IL-2 because (1) exogenous IL-2 corrects depressed allogeneic *in vitro* CTL in human lupus [9] and (2) defective *in vitro* IL-2 production is a frequent [10,11] but not uniform [12] observation in lupus patients.

Defective IL-2 production is also a hallmark of spontaneous murine lupus (reviewed in [13]) having been reported in the MRL/lpr, NZB, NZB/W, and BxSB models [14–16], further supporting the importance of this defect. Nevertheless, despite intense study for more than 25 years, the role of defective IL-2 production in lupus pathogenesis is unclear, particularly, whether it represents a primary pre-disposing defect, a secondary event, possibly compensatory aimed at reducing disease activity, or a combination of both primary and secondary events. This question is difficult to answer in humans because lupus patients can have an asymptomatic pre-clinical phase lasting years [17].

Similarly, in spontaneous lupus mice, the exact time of disease onset cannot be established prospectively with precision in individual mice, although a general time frame can be determined retrospectively. In contrast, in an induced model of lupus such as the parent-into-F1 (P → F1) model of lupus-like chronic graft-vs.-host disease (GVHD), the exact time of disease initiation is known with precision, thereby allowing a kinetic analysis of the very early events in disease. Results from this model and their implications regarding lupus T cell defects, particularly defective *in vitro* IL-2 production and cytotoxicity, are discussed below.

Insights into lupus-associated CTL and IL-2 defects from the P → F1 model of lupus

Background

A disease that strongly resembles human lupus can be induced in normal F1 mice by the transfer of parental T cells. For example, intravenous (or intraperitoneal) injection of homozygous parental strain CD4 T cells into immunologically normal unirradiated F1 mice results in the following features characteristic of human lupus: (1) humoral autoimmunity, initially anti-ssDNA followed at approximately 4 weeks by lupus-specific antibodies anti-dsDNA, anti-Sm, and anti-PARP; (2) immune complex glomerulonephritis; (3) lupus-like immunoglobulin and complement deposition in the skin; and (4) anti-RBC ab and autoimmune anemia [18–21]. Auto-antibodies characteristic of organ-specific autoimmunity, i.e. anti-thyroglobulin and anti-insulin, do not develop in this model.

Lupus-like disease results from donor CD4 T cell recognition of allogeneic MHC II on host B cells resulting in cognate help to all host B cells [22]. Activated host B cells that also encounter self-ag (e.g. DNA) mature into class-switched, IgG-secreting autoreactive B cells. Because murine T cells do not express MHC II, it is not strictly necessary that the recipient be an F1 but rather that (1) the donor and host differ by an MHC II disparity and (2) CD4 T cells are transferred in the donor inoculum. In an MHC II disparate GVHD, lupus-like

disease can result from the transfer of B6 CD4 T cells into either fully allogeneic MHC I + II disparate B6D2F1 hosts (no MHC I reactive donor cells are transferred) or the transfer of unfractionated B6 cells containing both CD4 and CD8 T cells into MHC II only disparate host such as the bm12 mutant B6 strain [20]. Donor CD8 T cells are not activated in the absence of an MHC I disparity.

The lupus-like phenotype is significantly altered if the donor inoculum contains parental CD8 T cells in addition to parental CD4 T cells, and the recipient is a fully allogeneic MHC I + II host. In this transfer, mice develop a phenotype that resembles acute GVHD seen in human bone marrow transplant recipients [23]. For example, the transfer of unfractionated B6 splenocytes containing both CD4 and CD8 T cells into MHC I + II disparate B6D2 F1 hosts results in the development of mature donor CD8 CTL effectors that eliminate host lymphocytes resulting in significant mortality at approximately 2–3 weeks [24]. Lastly, the transfer of donor CD8 T cells in the absence of donor CD4 T cells into F1 hosts that differ at least at MHC I does not result in a detectable phenotype due in large part to rejection of donor cells by host NK cells and possibly host T cells [25].

An exception to the above is the transfer of unfractionated DBA/2 parental splenocytes into B6D2F1 hosts. Despite the co-transfer of both CD4 and CD8 donor T cells into an MHC I + II disparate host, mice develop lupus long term rather than acute GVHD. This outcome results from defective DBA donor anti-host CD8 CTL expansion and effector maturation due to defects in both donor CD4 [26] and CD8 T cells [24,27]. Following sub-optimal maturation and expansion at day 10, donor DBA CD8 CTL contract and numbers are reduced. Donor DBA CD4 T cells, however persist [25] and the persisting donor CD4 chimerism promotes continued host B cell activation and auto-antibody production resulting in lupus as outlined above for MHC II disparate GVHD. Thus, donor CD4 T cells have a central role in driving B cell hyperactivity in this model just as pathogenic CD4 T cells are central to human lupus [28,29].

Surrogate markers allow GVHD phenotype designation at 2 weeks

Although acute and chronic GVHD were initially designated based on early or late mortality, respectively, these two phenotypes can be reliably differentiated at 2 weeks after donor cell transfer by a combination of flow cytometric, cytokine, and functional markers [25,30–32]. Briefly, donor CD4 T cells are absolutely required for both acute and chronic lupus-like GVHD induction and are responsible for disease initiation. It is the additional activation of donor CD8 T cells and their subsequent maturation into anti-host effector CTL that mediates the acute GVHD phenotype and differentiates it from chronic GVHD. At 10–14 days after donor cell transfer, acute GVHD mice exhibit engraftment of both donor CD4 and CD8 T cell subsets, striking elevations in IFN- γ , upregulation of Fas on donor and host T cells, evidence of donor anti-host CTL activity *in vitro* and *in vivo*, effector CD8 CTL maturation (increased perforin, granzyme, and FasL), and elimination of host cells, particularly B cells.

Although serum auto-antibodies are detected in acute GVHD mice initially, their level is low and transient. In contrast, at 10–14 days, chronic GVHD mice exhibit engraftment of donor CD4 T cells only, low-level elevation of IFN- γ , low-level Fas upregulation on T cells, and low-level evidence (if at all) of donor anti-host CTL. B cells are activated, expanded, and initially produce anti-ssDNA ab. Because host splenocytes are significantly reduced in acute GVHD and increased in chronic GVHD at 2 weeks, the 2-week phenotypes have been designated as cytotoxic and stimulatory, respectively [33].

Defective *in vitro* CTL and IL-2 production is an early feature in chronic lupus-like GVHD

Several P → F1 combinations have been assessed for *in vitro* T cell function in acute and chronic GVHD mice as measured by the ability of GVHD splenocytes to generate CTL effectors capable of lysing hapten-modified (TNP) self-targets or fully allogeneic targets following a 5-day *in vitro* sensitization phase [24,34]. CTL specific for TNP self-targets is a measure of ag-specific T cell responses and tests the capacity of both MHC II self-restricted CD4 T helper (Th) function for CTL and MHC I self-restricted CD8 CTL effector function. CTL killing of allogeneic targets also tests the self-restricted MHC I and II pathways but is a stronger response due to the higher precursor frequency of allo-specific vs. ag-specific T cells [35,36]. The presence of additional allogeneic antigen presenting cell (APC) also boosts the response by increasing the pathways of IL-2 production [37]. Because the ags used to test MHC self-restricted responses differ in humans and mice, we have designated this pathway as self plus X (S + X) and the allogeneic pathway as Allo.

Using this approach, assessment at 10–14 days after donor cell transfer, i.e. a time when GVHD phenotype is first detectable, revealed different results for acute GVHD mice compared with chronic GVHD mice. Acute GVHD mice exhibited complete loss of both S + X and Allo CTL responses, whereas chronic GVHD mice exhibited a selective loss of S + X response but preserved (albeit reduced in some cases) Allo CTL responses [24,34]. In chronic GVHD mice, the defective S + X CTL response could be restored by exogenous IL-2, consistent with a defect in *in vitro* IL-2 production. In contrast, defective CTL responses in acute GVHD mice to either S + X or Allo were not restored by IL-2 addition, consistent with a loss of precursor CTL.

To confirm the role of IL-2, *in vitro* IL-2 production was assessed in response to S + X or alloantigen in acute and chronic GVHD mice at 2 weeks after donor cell transfer [24]. Chronic GVHD mice exhibited near complete loss of *in vitro* IL-2 production for S + X but a relatively intact response to alloantigen, supporting the foregoing data that defective IL-2 production is responsible for the defective *in vitro* S + X CTL response in chronic GVHD. We have designated the combination of absent S + X response with preserved Allo response as -/+. Acute GVHD mice exhibited a complete loss of the IL-2 response to both S + X and Allo, mirroring the results of *in vitro* CTL generation and designated as -/-. The latter results indicate that both CD4 and CD8 T cells are defective in acute GVHD, consistent with the massive elimination of responding host T cells by donor CD8 CTL in day 14 acute GVHD spleens [24].

Interpretation of the -/+ pattern in chronic GVHD mice

In vitro IL-2 production for ag-driven responses involves a single MHC II self-restricted Th cell pathway that requires normally functioning responder CD4 T cells and MHC II syngeneic APC. In contrast, T cell production of IL-2 in response to alloantigen has three helper T cell pathways, i.e. an indirect pathway and two direct pathways [37]. The indirect Allo pathway is the MHC II self-restricted pathway also operative in S + X responses, i.e. alloantigen is processed and presented in the context of self-MHC II on self-APC to CD4 T cells. The two direct pathways result from direct presentation of alloantigen on allogeneic APC to responding T cells, allogeneic MHC II on stimulator APC presented directly to responding CD4 T cells and allogeneic MHC I on stimulator APC presented directly to responding CD8 T cells, resulting in IL-2 production by responder CD4 and CD8 T cells, respectively.

Cell depletion and add-back studies [24] demonstrated that the -/+ pattern in chronic GVHD mice is due to defective MHC II self-restricted CD4 Th cell function, indicating that the defect is not ag-specific but rather pathway-specific, i.e. both TNP and Allo responses

are lost when the response is solely dependent on this pathway. Moreover, the intact response of both direct Allo pathways indicates that normal allogeneic APC can overcome the IL-2 defect [24]. These results implicate a lupus-associated APC defect in mediating defective IL-2 production by Th cells in chronic GVHD mice although other defects are not excluded. These studies agree with previous work using PBL from human lupus patients demonstrating that *in vitro* allogeneic CTL responses are reduced but not absent [5], consistent with a loss of the S + X pathway and a $-/+$ phenotype.

Implications of the $-/+$ pattern in chronic GVHD for lupus pathogenesis

Because F1 mice are normal prior to donor cell transfer and would not otherwise develop lupus spontaneously, the $-/+$ defect in *in vitro* IL-2 and CTL appearing in chronic GVHD mice is, therefore, an induced or secondary defect. These results do not exclude the presence of an additional primary defect in some human lupus patients, but they clearly demonstrate that IL-2 defects can be secondary to active or ongoing lupus.

Second, defective *in vitro* IL-2 production is detectable as early as day 10 after donor cell transfer simultaneous with (a) maturation of donor effector CD4 Th cells, (b) peak host B cell expansion, and (c) significantly elevated levels of serum anti-ssDNA [25,32,34]. Thus, defective *in vitro* IL-2 production appears not just early in disease onset but appears in parallel with the development of *de novo* T cell-driven B cell hyperactivity and auto-antibody production, events that are weeks to months in advance of clinical evidence of renal disease [19,38]. Taken together, results from the P $\rightarrow$ F1 model of lupus indicate that the appearance of the $-/+$ pattern of IL-2 production is not only a secondary or induced defect, but it is also a sensitive and nearly simultaneous indicator of ongoing active lupus.

Defective IL-2 production in human lupus patients mirrors GVHD mice

The *in vitro* approach outlined above for GVHD mice was adapted for human studies, and PBL from healthy controls or SLE patients were assessed for IL-2 production as measured by testing the responses to S + X (i.e. recall ag tetanus) or human alloantigen. Both patients and controls were categorized as either $+/+$, $-/+$, or $-/-$ as described above. In a study of 8 healthy controls and 26 lupus patients, all controls were $+/+$, whereas lupus patients were heterogeneous in their IL-2 responses falling into one of three different patterns: (a) normal responses to both S + X and Allo ($+/+$), observed in 9 (35%) patients; (b) defective responses to S + X but intact Allo responses ($-/+$), observed in 12 (46%) SLE patients; and (c) defective responses to both S + X and Allo ($-/-$), observed in 5 (19%) SLE patients. There was no statistically significant correlation between immune response pattern and the use of immunosuppressants. Clinical activity was not prospectively assessed in this study and no correlations could be made.

The three *in vitro* Th cell pathways operative in allogeneic IL-2 responses, described above for murine splenocytes, are also operative in human PBL [39]. Cell depletion and add-back studies were performed and demonstrated [40] that by using APC-depleted allogeneic stimulator cells, $-/+$ SLE patients exhibited a defective CD4 MHC-self-restricted response to alloantigen (indirect pathway) as well as to S + X (influenza or tetanus). These results mimic those for $-/+$ chronic GVHD mice by confirming an ag-independent defect in the MHC self-restricted Th pathway and implicating an APC defect. Defective APC function of SLE PBL was further demonstrated as the inability irradiated PBL from selected $-/+$ SLE patients to stimulate an IL-2 response by normal APC-depleted PBL [40].

A larger study was subsequently performed in which IL-2 responses were categorized based on paired S + X (recall ags tetanus and influenza) and Allo responses as above. This study (1) confirmed the heterogeneity of defective IL-2 production in lupus patients and (2)

demonstrated that more severe defects were correlated with more severe disease activity. Specifically, of 150 SLE outpatients, Bermas et al. [41] demonstrated three response patterns in lupus patients but not in controls: (1) 76 (50%) patients responded to both S + X (recall ags influenza and/or tetanus) and Allo, (2) 62 (42%) patients responded to Allo but not to S + X (-/+), and (3) 12 (8%) patients did not respond to either stimulus (-/-). Greater *in vitro* dysfunction (-/-) was correlated with greater disease severity as measured by four scales of clinical activity. As in the previous study, *in vitro* dysfunction was not correlated with the use of immunosuppressants.

These studies on human lupus patients are in agreement with the P → F1 results and demonstrate that depressed *in vitro* IL-2 production is a lupus-associated defect. Moreover, the -/+ pattern in both human and P → F1 lupus involves defective APC function confirming the long-standing observation that APC are defective in human lupus [42].

IL-2 staging in other T cell-mediated conditions

The use of a pair of stimulants ranging from low precursor frequency responders and a single pathway of IL-2 production (i.e. S + X) to one of the greater precursor Th frequency of responders and multiple pathways of IL-2 production (i.e. Allo) allows the staging of Th cell dysfunction from mild (-/+) to significant (-/-). Prior to the discovery of HIV, high-risk homosexual males were reported to have defective *in vitro* S + X CTL responses [43]; however, further work demonstrated that some CTL non-responders to S + X could respond to Allo [44]. It was subsequently observed that the selective loss of S + X CTL characteristic of lupus-like GVHD mice was also seen in HIV antibody positive individuals (HIV+) and in some AIDS patients; however, a loss of both S + X and Allo responses was observed only in AIDS patients [45]. Using mitogen as a third stimulant, defects in *in vitro* Th cell function could now be stratified from mild (-/++) to severe (-/-/-). *In vitro* IL-2 was measured in 70 controls and 70 asymptomatic HIV+ individuals with normal CD4 T cell counts [46]. Responses were categorized as positive or negative to S + X (both influenza and tetanus), Allo, and PHA. All healthy controls exhibited a +/+ pattern, whereas four patterns of *in vitro* IL-2 production +/+, -/+, -/-+, and -/-/- were observed in HIV+ individuals. Moreover, HIV+ individuals exhibited a time-dependent progression from a stage responsive to all stimuli to a stage unresponsive to any of the stimuli with the earliest defect being a loss of S + X response. The most severe dysfunction (-/-/-) appeared to be a precursor to clinical AIDS. This suspicion was confirmed in a longitudinal prospective study (mean time = 3 years) in 335 HIV+ individuals demonstrating that the *in vitro* IL-2 response pattern predicted both survival time and time for progression to AIDS independently of CD4 T cell count [47].

The *in vitro* IL-2 staging approach was also applied to renal allograft recipients receiving immunosuppressive treatment (predominantly cyclosporine) and patients were categorized based on their response to S + X, Allo, and mitogen [48–50]. An intact S + X response (+/+ +) positively correlated with the risk of acute rejection and all of the patients who experienced acute graft rejection were in this group at the time of rejection. In contrast, no acute rejection episodes were observed in -/+ or -/-+ patients. Of note, all of the opportunistic infections occurred in patients with more pronounced immunosuppression, i.e. -/-+ suggesting that the loss of the *in vitro* Allo response represents clinically significant and excessive immunosuppression. No patients exhibited a loss of mitogen responsiveness (-/-/- pattern). Based on these results, it was concluded that periodic assessment of pathway-specific Th function is a sensitive index for detecting sub-therapeutic dosing of immunosuppressants and for assessing cyclosporine maintenance requirements.

These studies on both HIV+ individuals and allograft rejection patients using the *in vitro* IL-2 staging approach demonstrate that (1) it is possible to detect mild, moderate, or severe defects in T cell function, (2) the patterns have clinically significant implications, (3) the patterns involve intracellular and/or extracellular mechanisms other than simply loss of T cells, and (4) these studies are a direct extension of observations and mechanisms elucidated in the P → F1 model.

Mechanism of the $-/+$ IL-2 defect in murine lupus: Solely an APC defect?

Results from GVHD mice

As discussed above cell depletion and add-back studies indicate that defective APC function contributes to the $-/+$ IL-2 pattern in lupus-like GVHD mice. Complicating this interpretation, however, are the results of co-culture experiments demonstrating that splenocytes from day 14 lupus-like GVHD mice could induce a $-/+$ IL-2 and CTL defect in normal F1 splenocytes and that this effect was lost if GVHD splenocytes were irradiated prior to co-culture [24]. Attempts to further characterize the cell population responsible were inconclusive (C. S. Via, unpublished observations), and *in vitro* IL-2 staging and co-culture experiments were not performed in older lupus-like GVHD mice with more severe disease. Nevertheless, the presence of a radiosensitive suppressor cell population in lupus GVHD spleens raises the possibility that the $-/+$ pattern is multifactorial and involves a suppressor cell.

MRL/lpr spontaneous lupus mice also exhibit a $-/+$ Th response: CD4+ suppressor cell and APC implicated

A lupus-like disease spontaneously occurs in MRL/+ mice that is significantly accelerated in Fas defective MRL/lpr mice [51]. Testing of *in vitro* CTL and IL-2 production as outlined for GVHD mice was performed in both MRL/+ and MRL/lpr mice [52], and demonstrated that MRL/+ mice were $+/+$ at both 2 and 6 months of age, whereas only young (<2 months old) MRL/lpr mice were $+/+$ for CTL and IL-2 responses. By 4–6 months of age, MRL/lpr mice exhibited a $-/+$ IL-2 and CTL pattern similar to chronic GVHD mice. Just as in DBA → F1 lupus-like GVHD mice, the self-restricted response to Allo, i.e. APC-depleted Allo stimulators (Allo indirect pathway), was defective in older MRL/lpr mice supporting impaired APC function as a contributor to the defective MHC self-restricted T cell responses. Also, as seen in early DBA → F1 mice, co-culture experiments demonstrated that the $+/+$ pattern in MRL/+ mice could be converted into a $-/+$ pattern in the presence of old but not young MRL/lpr splenocytes. Surprisingly, phenotyping co-culture experiments demonstrated the presence of a CD4+ suppressor cell. Specifically, CD4+ cells, but not CD8+ cells, from older (6 months) MRL/lpr mice suppressed the CD4 MHC self-restricted IL-2 and CTL response to TNP-self and Allo (indirect pathway) of normal MRL/+ responders in co-culture. The suppressive effect of older MRL/lpr splenocytes was radiosensitive similar to P → F1 lupus-like GVHD mice.

These results taken in combination with those in lupus GVHD mice indicate that the $-/+$ *in vitro* Th pattern may be more complex than simply defective APC function. It is possible that defective APC function in these models of murine lupus and possibly human lupus is a consequence of a suppressor cell population that is both radiosensitive and CD4+. Although the CD4+ cell could be an APC, the radiosensitivity argues against this interpretation and supports CD4+ T cells as a suppressor population.

Remaining issues

Why don't lupus mice exhibit a $-/-$ defect as human SLE patients do?

As shown in Table I, chronic GVHD mice and MRL/lpr mice exhibit only the $-/+$ defect, whereas in human SLE (Table II) all three patterns of *in vitro* Th cell defects were observed with the $-/-$ pattern seen in patients with active/severe disease. The absence of a $-/-$ phenotype in lupus mice, while unexplained, may indicate that mice with very severe disease were not tested. Because lupus-like GVHD mice were only tested early in disease, it is possible they might progress to a $-/-$ phenotype as clinical disease worsens.

Arguing against this is the observation that older, sicker MRL/lpr mice exhibited only a $-/+$ phenotype and did not progress to a $-/-$ phenotype despite evidence of active disease. However, mortality in MRL/lpr mice is not necessarily due to lupus but can also be due to the massive lymphadenopathy. Thus, lupus severity in both chronic GVHD and the MRL/lpr mice tested may not have reached the same level of severity as seen in the $-/-$ human lupus patients. The possibility of a $-/-$ phenotype in older MRL/lpr has not been excluded from the testing of older mice with evidence of fulminant lupus. Moreover, the lpr mutation has been shown to result in abnormalities in CD4 T cell help and CD8 CTL contraction so that the MRL/lpr model may not accurately predict the long-term phenotype of chronic GVHD mice [30]. Last, in P $\rightarrow$ F1 lupus, disease severity is directly related to the number of donor cells injected and donor injection frequency [53]. The studies discussed above used a single injection of 80×10^6 DBA/2, just above the threshold of disease induction [31]. Resolution of this issue will require further studies using greater donor cell numbers to increase lupus-like GVHD severity and by testing mice both early and late in the course of clinical disease.

Reconciling the IL-2 staging approach with other reports in spontaneous lupus mice

The relatively mild $-/+$ defect reported for older MRL/lpr mice contrasts with the initial reports of defective IL-2 in these mice by other workers who demonstrated severe defects in response to the mitogen Con A [14–16]. It should be noted that in some studies, the indicator cells were not an IL-2-dependent cell line possibly altering the sensitivity but not the conclusions of the study. Second, the T cell mitogens Con A and PHA are dependent on APC function [54]; therefore, these studies do not preclude an APC defect.

Despite these differences in technical approach, Altman et al. [14] tested both Con A-induced IL-2 production and Allo-stimulated proliferation of old and young MRL/lpr mice, and although the Allo response of older MRL/lpr mice was reduced it was not absent (SI = 1.9). These findings were similar to our results with Allo-stimulated IL-2 and CTL responses and were consistent with a $-/+$ phenotype in which Allo responses may be reduced but are not absent. Thus, results using the *in vitro* Th staging approach do not conflict with previous studies in spontaneous lupus mice.

Significance of defective IL-2 production in lupus

Despite the clinical utility of defective *in vitro* IL-2 production as a marker for lupus activity, the implications of the defect remain unclear. For example, defective IL-2 production may be a beneficial compensatory mechanism that downregulates disease. Alternatively, defective IL-2 production may be a consequence of altered immune regulation that not only perpetuates disease but also contributes to the well-recognized increased incidence of infection in lupus patients [55], possibly as a result of defective IL-2-dependent CTL effector maturation. As cytokine and biologic therapeutic approaches advance, it may eventually be possible to correct IL-2 production *in vivo* in lupus patients. Prior to such therapeutic targeting, it will be imperative to determine whether the lupus-associated T cell defect in IL-2 production is beneficial or detrimental to patients.

Summary and conclusions

T cell defects are a well-described feature of both human and murine lupus and are likely multifactorial [56]. An unresolved question is whether defective T cell function in lupus is a primary pre-disposing event, a secondary event induced by disease activity, or a combination of both. Evidence from an induced model of lupus, the P → F1 model of chronic lupus-like GVHD, clearly demonstrates the secondary nature of the defect because impairment of both *in vitro* IL-2 and CTL responses can be induced in otherwise normal F1 mice early in the course of lupus-like disease and well in advance of clinical disease. Moreover, defective Th cell function was probed using a novel approach such that *in vitro* Th cell function was categorized based on the response to two stimuli: (1) the MHC self-restricted response, termed self + X and testing the ag-specific response of T cells and self-APC and (2) the allogeneic response, testing both the MHC self-restricted pathway and the ability of normal allogeneic APC to directly stimulate responder T cells and possibly correct defective SLE T cell function.

Using this approach, it was shown that lupus mice (both induced chronic GVHD mice and spontaneous MRL/lpr mice) exhibit a similar *in vitro* Th cell pattern, i.e. absent S + X response, but preserved allogeneic response termed -/+. In contrast, human lupus patients exhibited three possible response patterns, +/+, -/+, or -/-, with more severe *in vitro* T cell impairment correlated with more severe disease. Similarly, patients with other T cell-mediated conditions, i.e. HIV infection or renal allograft recipients, also exhibited more severe *in vitro* T cell impairment in association with greater disease activity or greater immunosuppression, respectively. In both human and murine lupus, the -/+ defect was associated with defective APC function and a radiosensitive suppressor cell. Additional studies in MRL/lpr mice indicated that the suppressor cell was CD4 positive raising the possibility that lupus-associated defects in APC function resulting in a -/+ Th cell pattern may be mediated by a suppressor cell, possibly a regulatory T cell.

Implications for lupus pathogenesis and future directions

A large number of parameters have been proposed for monitoring lupus disease activity to date, but no single test reliably predicts either disease flare prior to the onset of organ damage or predicts disease remission, which typically lags behind tissue injury parameters. The underlying abnormality in lupus is inappropriate T cell-driven, B cell hyperactivity resulting in the production of pathogenic auto-antibodies. Our results from P → F1 mice demonstrate that the loss of *in vitro* S + X IL-2 function (i.e. -/+ pattern) is an early event that develops in tandem with *de novo* T cell-driven, B cell hyperactivity and auto-antibody production, well before clinical evidence of renal disease. In human lupus patients, greater *in vitro* IL-2 impairment (-/- pattern) was associated with greater disease severity.

Taken together, these results demonstrate that lupus-associated defects in *in vitro* IL-2 and CTL responses are inducible defects that are parallel ongoing T cell-driven B cell hyperactivity. Moreover, the early appearance of *in vitro* IL-2 defects following lupus initiation and the correlation with disease severity indicates that *in vitro* assessment of IL-2 production is a sensitive measure of lupus activity. These results support the use of prospective monitoring of IL-2 responses in lupus patients. Although the *in vitro* IL-2 staging approach described herein using paired stimuli has demonstrated clinical utility, it may not be suitable for widespread clinical adaptation. Nevertheless, these results support further efforts to identify markers, possibly by flow cytometry, that correlate with the -/+ defect in IL-2 production and could be used in the clinical setting. Successful markers would allow not only the early institution of appropriate therapy to prevent organ damage but also the earliest possible tapering of agents, thereby reducing untoward pharmacological effects.

Lastly, we wish to underscore the critical role of the P → F1 model in serving as a springboard for the study of human T cell-mediated conditions, to include lupus. The elucidation of the mechanisms responsible for $-/+$ and $-/-$ patterns in P → F1 mice led to the successful adaptation of a similar approach in human diseases that allowed the staging of immune function and revealed important clinical implications regarding disease status. Very likely, this approach would not have been applied without the thorough mechanistic underpinning provided by the P → F1 results. Clearly, experimental lupus induction in the P → F1 model is artificial and does not mimic human etiologies, making it unlike the human conditions studied. Nevertheless, the appearance of similar Th response patterns in human and murine T cell-mediated conditions indicates that the underlying mechanisms involved are not disease specific but rather reflect important common immune responses and validate the use of the P → F1 model for future studies of T cell-mediated conditions.

References

1. Kammer GM, Perl A, Richardson BC, Tsokos GC. Abnormal T cell signal transduction in systemic lupus erythematosus. *Arthritis Rheum.* 2002; 46:1139–1154. [PubMed: 12115215]
2. Hahn BH, Bagby KK, Osterland CK. Abnormalities of delayed hypersensitivity in systemic lupus erythematosus. *Am J Med.* 1973; 55:25–31. [PubMed: 4736736]
3. Horwitz DA. Impaired delayed hypersensitivity in systemic lupus erythematosus. *Arthritis Rheum.* 1972; 15:353–359. [PubMed: 5046466]
4. Gottlieb AB, Lahita RG, Chiorazzi N, Kunkel HG. Immune function in systemic lupus erythematosus. Impairment of *in vitro* T-cell proliferation and *in vivo* antibody response to exogenous antigen. *J Clin Invest.* 1979; 63:885–892. [PubMed: 312809]
5. Tsokos GC, Balow JE. Cytotoxic responses to alloantigen in systemic lupus erythematosus. *J Clin Immunol.* 1981; 1:208–216. [PubMed: 19338096]
6. Perl A, Gonzalez-Cabello R, Lang I, Gergely P. Depressed natural and lectin-dependent cell-mediated cytotoxicity against adherent HEP-2 cells in patients with systemic lupus erythematosus. *Immunol Commun.* 1982; 11:431–440. [PubMed: 7169224]
7. Perl A, Gonzalez-Cabello R, Gergely P. Depressed effector activity of OKT4+ and OKT8+ T cell subsets in lectin-dependent cell-mediated cytotoxicity to HEP-2 cells in patients with systemic lupus erythematosus. *J Clin Lab Immunol.* 1984; 15:95–100. [PubMed: 6240539]
8. Tsokos, GC. Overview of cellular immune function in SLE. In: Lahita, RG., editor. *Systemic lupus erythematosus*. New York: Churchill Livingstone; 1992. p. 15-50.
9. Tsokos GC, Smith PL, Christian CB, Lipnick RN, Balow JE, Djeu JY. Interleukin-2 restores the depressed allogeneic cell-mediated lympholysis and natural killer cell activity in patients with systemic lupus erythematosus. *Clin Immunol Immunopathol.* 1985; 34:379–386. [PubMed: 3156017]
10. Alcocer-Varela J, Alarcon-Segovia D. Decreased production of and response to interleukin-2 by cultured lymphocytes from patients with systemic lupus erythematosus. *J Clin Invest.* 1982; 69:1388–1392. [PubMed: 6979554]
11. Linker-Israeli M, Bakke AC, Kitridou RC, Gendler S, Gillis S, Horwitz DA. Defective production of interleukin 1 and interleukin 2 in patients with systemic lupus erythematosus [SLE]. *J Immunol.* 1983; 130:2651–2655. [PubMed: 6222112]
12. Draeger AM, Swaak AJ, van den Brink HG, Aarden LA. T cell function in systemic lupus erythematosus: Normal production of and responsiveness to interleukin 2. *Clin Exp Immunol.* 1986; 64:80–87. [PubMed: 3488149]
13. Handwerger BS, Rus V, da Silva L, Via CS. The role of cytokines in the immunopathogenesis of lupus. *Springer Semin Immunopathol.* 1994; 16:153–180. [PubMed: 7716703]
14. Altman A, Theofilopoulos AN, Weiner R, Katz DH, Dixon FJ. Analysis of T cell function in autoimmune murine strains. Defects in production and responsiveness to interleukin 2. *J Exp Med.* 1981; 154:791–808. [PubMed: 6456321]
15. Dauphinee MJ, Kipper SB, Wofsy D, Talal N. Interleukin 2 deficiency is a common feature of autoimmune mice. *J Immunol.* 1981; 127:2483–2487. [PubMed: 6975325]

16. Wofsy D, Murphy ED, Roths JB, Dauphinee MJ, Kipper SB, Talal N. Deficient interleukin 2 activity in MRL/Mp and C57BL/6J mice bearing the *lpr* gene. *J Exp Med*. 1981; 154:1671–1680. [PubMed: 6975351]
17. Arbuckle MR, McClain MT, Rubertone MV, Scofield RH, Dennis GJ, James JA, Harley JB. Development of autoantibodies before the clinical onset of systemic lupus erythematosus. *N Engl J Med*. 2003; 349:1526–1533. [PubMed: 14561795]
18. Gleichmann E, Gleichmann H. Pathogenesis of graft-versus-host reactions (GVHR) and GVH-like diseases. *J Invest Dermatol*. 1985; 85:115s–120s. [PubMed: 3159804]
19. Gleichmann E, Van Elven EH, Van der Veen JP. A systemic lupus erythematosus [SLE]-like disease in mice induced by abnormal T–B cell cooperation. Preferential formation of autoantibodies characteristic of SLE. *Eur J Immunol*. 1982; 12:152–159. [PubMed: 6978818]
20. Eisenberg R. The chronic graft-versus-host model of systemic autoimmunity. *Curr Dir Autoimmun*. 2003; 6:228–244. [PubMed: 12408055]
21. Grader-Beck T, Casciola-Rosen L, Lang TJ, Puliaev R, Rosen A, Via CS. Apoptotic splenocytes drive the autoimmune response to poly[ADP-ribose] polymerase 1 in a murine model of lupus. *J Immunol*. 2007; 178:95–102. [PubMed: 17182544]
22. Morris SC, Cheek RL, Cohen PL, Eisenberg RA. Autoanti-bodies in chronic graft versus host result from cognate T–B interactions. *J Exp Med*. 1990; 171:503–517. [PubMed: 2303783]
23. Niculescu F, Niculescu T, Nguyen P, Puliaev R, Papadimitriou JC, Gaspari A, Rus H, Via CS. Both apoptosis and complement membrane attack complex deposition are major features of murine acute graft-vs-host disease. *Exp Mol Pathol*. 2005; 79:136–145. [PubMed: 15979610]
24. Via CS, Sharrow SO, Shearer GM. Role of cytotoxic T lymphocytes in the prevention of lupus-like disease occurring in a murine model of graft-vs-host disease. *J Immunol*. 1987; 139:1840–1849. [PubMed: 2957440]
25. Puliaev R, Puliaeva I, Welniak LA, Ryan AE, Haas M, Murphy WJ, Via CS. CTL-promoting effects of CD40 stimulation outweigh B cell-stimulatory effects resulting in B cell elimination and disease improvement in a murine model of lupus. *J Immunol*. 2008; 181:47–61. [PubMed: 18566369]
26. De Wit D, Van Mechelen M, Zanin C, Doutrelepon JM, Velu T, Gerard C, Abramowicz D, Scheerlinck JP, De Baetselier P, Urbain J, et al. Preferential activation of Th2 cells in chronic graft-versus-host reaction. *J Immunol*. 1993; 150:361–366. [PubMed: 8419469]
27. Tschetter JR, Mozes E, Shearer GM. Progression from acute to chronic disease in a murine parent-into-F1 model of graft-versus-host disease. *J Immunol*. 2000; 165:5987–5994. [PubMed: 11067962]
28. Mohan C, Adams S, Stanik V, Datta SK. Nucleosome: A major immunogen for pathogenic autoantibody-inducing T cells of lupus. *J Exp Med*. 1993; 177:1367–1381. [PubMed: 8478612]
29. Crow MK, DelGiudice-Asch G, Zehetbauer JB, Lawson JL, Brot N, Weissbach H, Elkon KB. Autoantigen-specific T cell proliferation induced by the ribosomal P2 protein in patients with systemic lupus erythematosus. *J Clin Invest*. 1994; 94:345–352. [PubMed: 7913711]
30. Puliaeva I, Puliaev R, Shustov A, Haas M, Via CS. Fas expression on antigen-specific T cells has costimulatory, helper, and down-regulatory functions *in vivo* for cytotoxic T cell responses but not for T cell-dependent B cell responses. *J Immunol*. 2008; 181:5912–5929. [PubMed: 18941180]
31. Via CS, Shearer GM. Murine graft-versus-host disease as a model for the development of autoimmunity. Relevance of cytotoxic T lymphocytes. *Ann N Y Acad Sci*. 1988; 532:44–50. [PubMed: 3263074]
32. Rus V, Svetic A, Nguyen P, Gause WC, Via CS. Kinetics of Th1 and Th2 cytokine production during the early course of acute and chronic murine graft-versus-host disease. Regulatory role of donor CD8 + T cells. *J Immunol*. 1995; 155:2396–2406. [PubMed: 7650373]
33. Puliaeva I, Puliaev R, Via CS. Therapeutic potential of CD8 + cytotoxic T lymphocytes in SLE. *Autoimmun Rev*. 2009; 8:219–223. [PubMed: 18725326]
34. Moser M, Mizuochi T, Sharrow SO, Singer A, Shearer GM. Graft-vs-host reaction limited to a class II MHC difference results in a selective deficiency in L3T4 + but not in Lyt- 2+ T helper cell function. *J Immunol*. 1987; 138:1355–1362. [PubMed: 2949013]

35. Felix NJ, Allen PM. Specificity of T-cell alloreactivity. *Nat Rev Immunol.* 2007; 7:942–953. [PubMed: 18007679]
36. Lindahl KF, Wilson DB. Histocompatibility antigen-activated cytotoxic T lymphocytes. II. Estimates of the frequency and specificity of precursors. *J Exp Med.* 1977; 145:508–522. [PubMed: 233899]
37. Singer A, Munitz TI, Golding H, Rosenberg AS, Mizuochi T. Recognition requirements for the activation, differentiation and function of T-helper cells specific for class I MHC alloantigens. *Immunol Rev.* 1987; 98:143–170. [PubMed: 2958403]
38. Lang TJ, Nguyen P, Papadimitriou JC, Via CS. Increased severity of murine lupus in female mice is due to enhanced expansion of pathogenic T cells. *J Immunol.* 2003; 171:5795–5801. [PubMed: 14634088]
39. Via CS, Tsokos GC, Stocks NI, Clerici M, Shearer GM. Human *in vitro* allogeneic responses. Demonstration of three pathways of T helper cell activation. *J Immunol.* 1990; 144:2524–2528. [PubMed: 1969449]
40. Via CS, Tsokos GC, Bermas B, Clerici M, Shearer GM. T cell-antigen-presenting cell interactions in human systemic lupus erythematosus. Evidence for heterogeneous expression of multiple defects. *J Immunol.* 1993; 151:3914–3922. [PubMed: 8376810]
41. Bermas BL, Petri M, Goldman D, Mittleman B, Miller MW, Stocks NI, Via CS, Shearer GM. T helper cell dysfunction in systemic lupus erythematosus [SLE]: Relation to disease activity. *J Clin Immunol.* 1994; 14:169–177. [PubMed: 7929693]
42. Kuntz MM, Innes JB, Weksler ME. The cellular basis of the impaired autologous mixed lymphocyte reaction in patients with systemic lupus erythematosus. *J Clin Invest.* 1979; 63:151–153. [PubMed: 153913]
43. Shearer GM, Payne SM, Joseph LJ, Biddison WE. Functional T lymphocyte immune deficiency in a population of homosexual men who do not exhibit symptoms of acquired immune deficiency syndrome. *J Clin Invest.* 1984; 74:496–506. [PubMed: 6611354]
44. Shearer GM, Salahuddin SZ, Markham PD, Joseph LJ, Payne SM, Kriebel P, Bernstein DC, Biddison WE, Sarngadharan MG, Gallo RC. Prospective study of cytotoxic T lymphocyte responses to influenza and antibodies to human T lymphotropic virus-III in homosexual men. Selective loss of an influenza-specific, human leukocyte antigen-restricted cytotoxic T lymphocyte response in human T lymphotropic virus-III positive individuals with symptoms of acquired immunodeficiency syndrome and in a patient with acquired immunodeficiency syndrome. *J Clin Invest.* 1985; 76:1699–1704. [PubMed: 2997287]
45. Shearer GM, Bernstein DC, Tung KS, Via CS, Redfield R, Salahuddin SZ, Gallo RC. A model for the selective loss of major histocompatibility complex self-restricted T cell immune responses during the development of acquired immune deficiency syndrome (AIDS). *J Immunol.* 1986; 137:2514–2521. [PubMed: 3489767]
46. Clerici M, Stocks NI, Zajac RA, Boswell RN, Lucey DR, Via CS, Shearer GM. Detection of three distinct patterns of T helper cell dysfunction in asymptomatic, human immunodeficiency virus-seropositive patients. Independence of CD4+ cell numbers and clinical staging. *J Clin Invest.* 1989; 84:1892–1899. [PubMed: 2574188]
47. Dolan MJ, Clerici M, Blatt SP, Hendrix CW, Melcher GP, Boswell RN, Freeman TM, Ward W, Hensley R, Shearer GM. *In vitro* T cell function, delayed-type hypersensitivity skin testing, and CD4+ T cell subset phenotyping independently predict survival time in patients infected with human immunodeficiency virus. *J Infect Dis.* 1995; 172:79–87. [PubMed: 7797948]
48. Muluk SC, Clerici M, Via CS, Weir MR, Kimmel PL, Shearer GM. Correlation of *in vitro* CD4+ T helper cell function with clinical graft status in immunosuppressed kidney transplant recipients. *Transplantation.* 1991; 52:284–291. [PubMed: 1678559]
49. Schulick RD, Muluk SC, Clerici M, Bermas BL, Via CS, Weir MR, Shearer GM. Value of *in vitro* CD4+ T helper cell function test for predicting long-term loss of human renal allografts. *Transplantation.* 1994; 57:480–482. [PubMed: 7906443]
50. Schulick RD, Weir MB, Miller MW, Cohen DJ, Bermas BL, Shearer GM. Longitudinal study of *in vitro* CD4+ T helper cell function in recently transplanted renal allograft patients undergoing tapering of their immunosuppressive drugs. *Transplantation.* 1993; 56:590–596. [PubMed: 8105569]

51. Cohen PL, Eisenberg RA. Lpr and gld: Single gene models of systemic autoimmunity and lymphoproliferative disease. *Annu Rev Immunol.* 1991; 9:243–269. [PubMed: 1910678]
52. Via CS, Shearer GM. Functional heterogeneity of L3T4+ T cells in MRL-lpr/lpr mice. L3T4+ T cells suppress major histocompatibility complex-self-restricted L3T4+ T helper cell function in association with autoimmunity. *J Exp Med.* 1988; 168:2165–2181. [PubMed: 2974064]
53. Van Rappard-Van Der Veen FM, Radaszkiewicz T, Terraneo L, Gleichmann E. Attempts at standardization of lupus-like graft-vs-host disease: Inadvertent repopulation by DBA/2 spleen cells of H-2-different nonirradiated F1 mice. *J Immunol.* 1983; 130:2693–2701. [PubMed: 6602176]
54. Shevach, EM. Macrophages and other accessory cells. In: Paul, WE., editor. *Fundamental immunology.* New York: Raven Press; 1984. p. 71-107.
55. Iliopoulos AG, Tsokos GC. Immunopathogenesis and spectrum of infections in systemic lupus erythematosus. *Semin Arthritis Rheum.* 1996; 25:318–336. [PubMed: 8778988]
56. Crispin JC, Kyttaris V, Juang YT, Tsokos GC. Systemic lupus erythematosus: New molecular targets. *Ann Rheum Dis.* 2007; 66(Suppl 3):iii65–iii69. [PubMed: 17934100]

Table 1

Selective loss of *in vitro* IL-2 and CTL responses to S + X are a feature of both induced and spontaneous murine lupus.

Stimulant*	Control F1†	Chronic GVHD	MRL/+2-6 months	MRL/lpr 2 months	MRL/lpr 6 months
S + X	+	-	+	+	-
Allo	+	+	+	+	+

* *In vitro* IL-2 and CTL responses to S + X (hapten-modified autologous APC) or to alloantigen were measured as described [24,34] and are denoted as either detectable (+) or absent (-).

† Groups tested are controls (un-injected normal F1 mice), chronic GVHD mice at 10-14 days of disease, or MRL/+ and MRL/lpr at the ages shown.

Table II

Severity of defective *in vitro* IL-2 production in human SLE patients correlates with disease activity.

Stimulant [*]	Healthy control [†]	SLE less	SLE moderate	SLE more
S + X	+	+	–	–
Allo	+	+	+	–
Mitogen	+	+	+	+

^{*} *In vitro* IL-2 production of human PBL was measured in response to S + X (recall ags influenza or tetanus), human alloantigen, or the mitogen PHA and responses denoted as detectable or absent as described [41];

[†] Groups shown: healthy human control PBL or PBL from SLE patients whose mean disease activity per group was less severe, intermediate, or more severe as described [41].