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EDITORIAL

Pre-clinical screening of immunomodulatory compounds using the parent-into-F1 model☆

Activation of the innate immune system by toll-like receptors (TLR) provides an early response to pathogens and shapes the subsequent antigen-specific adaptive immune response [1]. Unmethylated cytosine-guanine dinucleotide (CpG) motifs present in bacterial DNA, but not vertebrate DNA, can be detected by Toll like receptor 9 (TLR9) — bearing DC and B cells resulting in a pro-inflammatory Th1 response [2–4]. Synthetic oligodeoxynucleotides (ODN) expressing these CpG motifs exhibit similar in vivo biologic activity and have been shown to reduce disease severity for selected pathogens, to promote vaccine responses and to enhance anti-tumor responses (reviewed in Ref. [4]). Conversely, suppressive DNA sequences have also been identified [4]. TTAGGG elements in mammalian telomeric DNA and synthetic ODNs containing these motifs can inhibit pro-inflammatory and Th1 cytokine production [5] and have been reported as beneficial in autoimmune conditions such as collagen-induced arthritis [6] and lupus nephritis [7].

Microsatellite (MS) DNA is composed of short tandemly repeated DNA sequences widely distributed in the human genome. MS DNA has been used as a molecular marker for DNA fingerprinting analyses and alterations in MS DNA triplet repeats have been associated with several hereditary diseases, primarily neurological (reviewed in Ref. [8]). Recently, ODNs with sequences based on MS DNA have been shown to exhibit inhibitory effects on the immune response [9]. One of these MS DNA-mimicking ODNs with CCT repeats (compound SATO5f) was reported to down regulate TLR7/9-dependent interferon alpha (IFN-a) production [9] raising the possibility that SATO5f and similar compounds may be beneficial in conditions where increased IFN-a has a pathogenic role e.g., lupus [10]. Data supporting this idea are presented in the current issue where in the article by He et al. [11], in vivo administration of SATO5f is effective in preventing lupus-like renal disease occurring in an induced model of murine lupus, the parent-into-F1 (p→F1) model of chronic graft-vs.-host disease (GVHD). Administration of SATO5f was administered twice weekly i.p. beginning at the time of first donor cell transfer and continued for 8–10 weeks resulted in significant reduction of autoantibodies and ICGN severity compared to untreated chronic GVHD mice. An intermittent dosing scheme was also effective. This work not only supports the potential clinical application of a new class of immunomodulatory compounds in lupus but it also underscores the usefulness of animal models in screening for biologic agents with therapeutic potential.

Improvement in lupus-like ICGN by a candidate compound is the gold standard in pre-clinical animal testing. In this regard, both spontaneous and induced murine lupus models have been valuable. Although spontaneous lupus models such as the MRL/lpr and female NZBxWF1 have been widely used, a major drawback is that severe ICGN may require ~6 months for development. In the p→F1 model, lupus-like ICGN severity varies among the strains used [12–14] however some strain combinations can exhibit ICGN as early as 8–12 weeks after donor cell transfer. Moreover, unlike spontaneous lupus models, in the p→F1 model, the exact time of disease onset, the initiating antigen and the pathogenic T cells (donor CD4 T cells) are all known and the latter are of sufficient frequency that they are readily identified by flow cytometry without the need for custom tetramers. Together, these features have allowed much of the kinetics of acute and chronic GVHD pathogenesis in p→F1 mice to be elucidated (reviewed in Ref. [14]). For example, acute GVHD seen following the transfer of C57Bl/6 (B6) splenocytes into (B6×DBA/2)F1 mice (B6→BDF1) results from donor CD4 and CD8 T cell recognition of host MHC II and MHC I alloantigens respectively. Donor anti-host CD8 CTL specific for host MHC I eliminates host lymphocytes and induces immunodeficiency rather than autoimmunity and ICGN. By contrast, chronic GVHD can be induced in B6→BDF1 mice using donor splenocytes depleted of CD8 T cells indicating that chronic GVHD results solely from donor CD4 T cell activation in response to host allogeneic MHC II. The resulting cognate CD4 T cell help to host B cells induces host lymphocyte expansion, lupus specific autoantibodies and, depending on the strain combinations, ICGN [15,16]. Moreover, acute and chronic GVHD phenotypes can be distinguished by flow cytometry at

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2 weeks after donor cell transfer [17] allowing rapid identification of promising compounds with immunomodulatory potential.

In the current study by He et al., the authors used the Balb/c→CB6F1 strain combination and administered multiple transfers of T cell-containing lymphocytes from spleen, lymph node and thymus. This approach has the advantage of inducing a relatively rapid (8–10 weeks) lupus-like ICGN as opposed to that seen following a single transfer of unfractionated Balb/c splenocytes which can require 6 months for ICGN [18]. However a single transfer of unfractionated splenocytes can induce ICGN at 10–12 weeks if female DBA/2 (DBA) donors (containing ~12–14×10^6 CD4 T cells) are transferred into female BDF1 hosts [19]. Unlike chronic GVHD seen using CD8 depleted B6 donor cells and BDF1 hosts, chronic GVHD in both the BALB/c→CB6F1 and the DBA→BDF1 chronic GVHD is seen despite the transfer of donor CD8 T cells [18,20]. For both p→F1 combinations, the donor anti-host CD8 CTL response is impaired resulting in chronic rather than acute GVHD due to an intrinsic and possibly an extrinsic CD8 T cell defect. For example, both DBA and Balb/c donor CD8 T cells have a significant reduction in the anti-allogeneic CD8 precursor CTL frequency compared to that of B6 CD8 T cells [18,20]. Additionally, both DBA and Balb/c CD4 T cells are strong IL-4 and weak IFN-g producers compared to B6 mice where the reverse trend is observed [21–24] raising the possibility that defective donor anti-host CD8 CTL in DBA→BDF1 and Balb/C→CB6F1 mice is secondary to sub optimal CD4 T cell help. Although the suboptimal DBA CD8 CTL cell response is not required for donor CD4 T cell driven lupus-like disease in DBA→BDF1 mice, we have recently observed that DBA donor CD8 T cells are critical for sex-based differences (i.e. greater female severity) in both long term ICGN [19] and in two-week donor CD4 T cell engraftment [25] indicating a novel role of CD8 T cells in shaping sex based differences in immune function.

An important feature of the p→F1 model is that the recipient is normal (i.e. non lupus-prone) and immunologically intact prior to donor cell transfer as opposed to GVHD models for bone marrow transplantation where the recipient is irradiated. Thus, in addition to modeling human lupus, the p→F1 model is also an in vivo model of a normal ag-driven T cell driven response. Acute GVHD in B6→BDF1 mice is a model for normal CD8 CTL development whereas chronic GVHD in DBA→BDF1 mice is a model for CD4 T cell activation in the setting of a failed or impaired in vivo CD8 CTL response. Together these two combinations have pre-clinical value in the testing of biologically active compounds in addition to testing for potential lupus-specific therapeutics. For example, B6→BDF1 mice are useful for the screening of compounds that selectively block CD8 CTL maturation thereby converting disease phenotype from acute to chronic. Such compounds could be of use in clinical situations where CD8 CTL are undesirable e. g., preventing early allograft rejection (reviewed in Ref. [14]).

Conversely, the DBA CD8 T cell abnormality can be exploited in DBA→BDF1 mice to screen for agents with CD8 CTL promoting properties thereby converting disease phenotype from chronic to acute. Such compounds could be useful in enhancing anti-tumor or anti-viral responses and may possibly be useful in lupus. Promising candidates must then be further examined in long term studies to ascertain whether the two-week phenotype is maintained and that mice do not revert to lupus-like disease long term. If reversion is seen, this may be an indication of the need for either additional doses of the test compound or possibly a failure of CD8 and/or memory T cells (see below).

Using this approach, we have observed that chronic GVHD can be converted to acute GVHD in DBA→F1 mice by the in vivo administration of either: a) rIL-12, b) a blocking anti-CD80 mAb, or c) an agonist anti-CD40 mAb. Although all three agents induce a strong DBA donor CD8 CTL and convert two-week phenotype to acute GVHD, the mechanism involved is different in each case. For example, rIL-12, a product of the innate immune response, is well known as promoter of CD8 CTL [26]. Acute GVHD is induced in DBA→BDF1 mice if rIL-12 is given during the first 5 days after donor cell transfer however the effect is lost if administration is delayed until day 7. Thus, rIL-12 administration likely circumvents the DBA CD8 CTL defect by providing requisite amounts of signal 3 to newly activated donor CD8 T cells [27].

By contrast, in vivo anti-CD80 mAb treatment of DBA→BDF1 mice promotes donor CD8 CTL and induces acute GVHD not by enhancing initial activation but by impairing CD8 CTL effector downregulation. Upregulation of CD80 and CD86 is well described on activated T cells [28–31]. Similarly, in p→F1 mice, donor T cell upregulation of CD80, more so than CD86, is seen by ~day 10 and acts to limit their expansion [32]. Confirming this view, donor T cells defective in CD80 exhibit enhanced induction of acute GVHD due to greater peak CD8 CTL effector numbers [32]. Treatment of DBA→BDF1 mice with blocking anti-CD80 mAb but not anti-CD86 mAb boosts peak expansion of donor CD8 T cell effectors by blocking CD80 mediated effector limitation [33] resulting in acute GVHD. These results support the idea that anti-CD80 mAb may be a useful immunotherapeutic adjunct in clinical situations where CD8 CTL promotion is desired such as suboptimal responses to tumors or viruses.

Lastly, a single dose of agonist anti-CD40 mAb at the time of donor cell transfer in DBA→BDF1 mice exhibits both T cell and B cell stimulating properties and licenses APC thereby bypassing the need for donor CD4 T cell help for CD8 CTL [34]. In the short term, anti-CD40 treated DBA→BDF1 mice exhibit acceleration of both donor CD8 CTL development and acute GVHD phenotype compared to that seen in B6→BDF1 mice (i.e. conventional CD4 T cell help for CD8 CTL). By 12 weeks, however, anti-CD40 treated DBA→BDF1 mice exhibit serological reversion to chronic GVHD in conjunction with reduced numbers donor CD8 T cells and increased numbers of host B cells compared to B6→BDF1 mice which maintain the acute GVHD phenotype. Because memory CD8 T cells require CD4 T cell help during priming [35], it is possible that serologic reversion to lupus reflects a lack of memory CD8 T cells in anti-CD40 mAb treated DBA→BDF1 mice. Although these results do not necessarily support a therapeutic role for agonist anti-CD40 mAb in lupus, they raise the novel possibility that CD8 T cells, possibly memory, are important in maintaining lupus remission. Further studies will be necessary to confirm these results however, they are consistent with work in spontaneous lupus models demonstrating an important role for CD8 T cells in downregulating humoral autoimmunity either as CD8 Tregs [36] or as Qa-restricted CD8 T cells which suppress T follicular helper activity to autoreactive B cells [37,38].

With the advent of DNA based compounds with the potential to modify both innate and adaptive immune responses, a new

direction in the treatment of immune mediated conditions is possible in humans. Mouse models will continue to play an important role in identifying promising compounds and the P→F1 model offers a number of experimental advantages. It will be of interest to determine whether SATO5f and other MS DNA based ODNs act to improve lupus by promoting CD8 T cells, inhibiting CD4 help for B cells or a use a different mechanism. Moreover, in defining a compounds mechanism of action, it is possible that underlying mechanisms of disease pathogenesis may also be revealed.

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