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Mixed Hematopoietic Chimerism Allows Cure of Autoimmune Glomerulonephritis: Its Potential and Risks

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1. Introduction

Patients with severe autoimmune lupus glomerulonephritis that is resistant to immunosuppressive therapy need alternative treatment. Recently, bone marrow transplantation (BMT) has been proposed as a potential therapy for refractory autoimmune disease. BMT involves the administration of hematopoietic stem cells, which are self-renewing and capable of giving rise to all mature hematopoietic cell types and possibly some non-hematopoietic cell types. The etiologic and pathogenic bases of many autoimmune diseases ultimately reside in the self-renewing hematopoietic stem cell population. Therefore, the effects of BMT as a treatment for and/or preventive measure against these autoimmune diseases have been investigated extensively (Sykes&Nicolic, 2005). Studies in animal models have shown that the transfer of hematopoietic stem cells can reverse the autoimmune state. The induction of fully allogeneic bone marrow (BM) chimerism, however, is fraught with difficulties. Each of the various methods of inducing fully allogeneic BM chimerism through hematopoietic cell transplantation (HCT) requires a different set of conditions, such as host T cell depletion, donor myeloablation, major histocompatibility complex (MHC) fully matched donor BM, or lethal dose of total body irradiation (TBI). Meeting these conditions is usually a burden on the recipient. Moreover, fully allogeneic BM chimerism is always associated with risks of graft versus host disease (GVHD) and immunodeficiency, which make it less practical for clinical application.

Accordingly, the induction of mixed allogeneic BM chimerism has been proposed as a treatment for autoimmune disease. Mixed chimerism refers to a state in which allogeneic donor hematopoietic cells coexist with recipient cells in host bone marrow.

In this paper, the advantages of inducing mixed BM chimerism are summarized and a process for inducing peripheral/central tolerance is introduced. Several mechanistic pathways which are thought to be involved in reversing the autoimmune state are then described. Based on our original data, we propose one possible mechanism in which newly developed donor T cells, which have been positively selected in the host thymus and restricted host MHC, are able to regulate auto-reactive B cells through T cell receptor (TCR)/MHC interaction. Finally, we discuss the potential risks associated with fully MHC-

mismatched allogeneic mixed chimerism. This information will help to determine the role that HCT can play in the treatment of autoimmune glomerulonephritis.

2. Bone marrow mixed chimerism

2.1 What is the advantage of mixed chimerism?

Mixed chimerism refers to a state in which allogeneic donor hematopoietic cells coexist with recipient cells in host bone marrow, whereas fully allogeneic chimerism refers to a state in which donor hematopoietic cells completely replace recipient cells.

It is known that fully allogeneic chimeras transplanted from a donor with fully mismatched MHC usually reject donor BM, or experience severe GVHD. Even if donor BM cells were safely engrafted in host BM, the resulting fully MHC-mismatched chimeras would develop immunodeficiency. In fully allogeneic chimeras, all mature T cells are supposed to be restricted to the host MHC type, irrespective of their own genetic background. This occurs because thymocytes, the precursors of mature T cells, are positively selected for weak reactivity to the self-peptide/MHC complex in the host thymus; this positive selection is mediated only by thymic cortical epithelial cells and not by bone marrow-derived cells. Therefore, in the periphery, all TCRs have certain affinity to host MHC molecules but not to donor MHC molecules. Thus, if the donor MHC is fully mismatched with the host MHC, there are no peripheral T cells which can interact with peripheral B cells differentiated from donor hematopoietic stem cells which generate donor-type MHC. This is the cause of deficiency in humoral immunity in fully MHC-mismatched allogeneic chimerism (Janeway et al., 2001).

In mixed chimerism, on the other hand, TCR/MHC interactions are at least partially maintained, because B cells differentiated from recipient hematopoietic stem cells are still being generated. Moreover, during intrathymic development, thymocytes that have high affinity to self MHC molecules are deleted from the repertoire in a process known as negative selection. Thymocytes from both the recipient and the donor mature on the thymic epithelium expressing MHC molecules with the recipient haplotype. Nevertheless, the repertoire of T cells, which react with high affinity to MHC molecules with the donor haplotype, eliminated in mixed chimera. This implies that bone marrow-derived cells must be able to induce negative selection. Actually, negative selection in the thymus can be mediated by several different cells. The most important of these are the BM-derived dendritic cells and macrophages. In mixed chimera, the dendritic cells and macrophages differentiated from both donor and host hematopoietic stem cells are located in the thymus, where they eliminate T cells with strong reactivity to self-peptides on both donor and host MHC; thus donor- and host-specific tolerance to each other is established.

To summarize, mixed chimerism offers several advantages over full chimerism as a means of treating autoimmune disease:

1. Mixed chimeras exhibit superior immune-competence across complete MHC barriers. Mixed chimeras possess certain populations of antigen presenting cells (APCs) and B cells which express host-type MHC molecules in the periphery, whereas mixed chimeras exhibit normal humoral and cellular immune responses.
2. In mixed chimeras, dendritic cells and macrophages differentiated from both the recipient and the donor hematopoietic stem cells locate to the thymus where they delete both host-reactive and donor-reactive T cells through negative selection, resulting in a

peripheral T cell repertoire that is tolerant toward both donor and host cells. Therefore, GVHD, one of the most important complications of allogeneic BMT, is not seen in mixed chimeras.

3. Mixed chimerism can be achieved through non-myeloablative regimens, which are generally less toxic than the myeloablative regimens necessary to induce full BM chimerism (this point will be discussed in detail in the next section).

2.2 How is mixed chimerism induced?

As explained above, once specific tolerance is established, the state of mixed chimerism is thought to be stable. The difficulty in establishing stable mixed chimerism lies in blocking the first attack of host peripheral T cells on donor bone marrow stem cells until “tolerized” T cells are renewed in the host thymus. Because host T cells play a dominant role in the rejection of allografts, several methods of deleting host T cells through the injection of various lymphocyte-deleting antibodies along with either total body irradiation or immunosuppressive drugs have been attempted (Tomita et al, 1996. Nikolic et al, 2000). These regimens enabled BM engraftment but were a burden on recipients and frequently made them susceptible to infection. The toxicity of the necessary conditioning regimens has precluded the use of this approach in clinical transplantation.

Another method of inducing allogeneic tolerance involves the temporary inhibition of co-stimulatory interaction between APCs and T cells by injecting blocking antibodies. This method works because T cell activation without proper co-stimulation can induce a state of antigen-specific non-responsiveness (Fig.1).

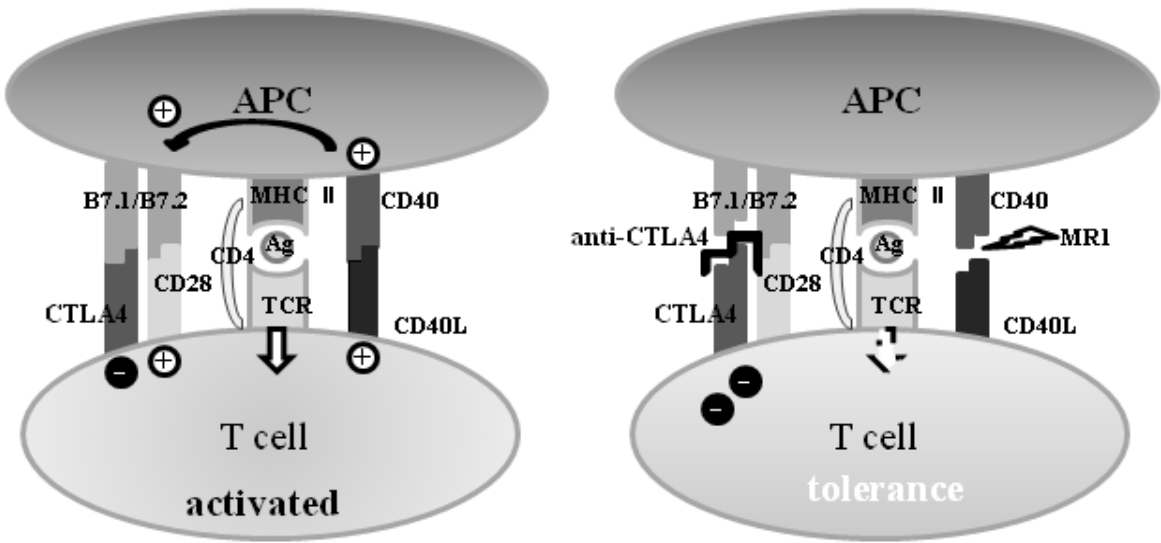


Fig. 1. Activation of naïve T cells requires co-stimulation. Binding of the peptide/MHC complex by the TCR and the CD4 or CD8 co-receptor transmits the first signal to the T cell. Activation of naïve T cells requires a second signal, namely, the ligation of B7 molecules (B7.1/B7.2) and CD28, which stimulates the clonal expansion of naïve T cells. Binding of CD40L by CD40 plays a central role effector function in the full differentiation of T cells. This ligation also activates APCs to express B7 molecules (left). Stimulation of CTLA4 with anti-CTLA4 Ab and/or Blocking CD40/CD40L by MR1 induces antigen-specific T cell tolerance (right).

Recently, Takeuchi et al. have shown that administration of MHC-mismatched donor bone marrow to mice receiving 3Gy TBI one day before BMT and a single injection of Hamster- anti-mouse CD40L monoclonal antibody (MR1, hybridoma) intraperitoneally (i.p.) with BMT permitted the induction of permanent mixed chimerism and tolerance without T cell depletion (Y. Takeuchi et al., 2004). This regimen is quite simple and less toxic than the alternatives, because 3Gy TBI is nonlethal and does not require MHC matching. Therefore we have adopted this regimen for treatment of autoimmune disease in systemic lupus erythematosus (SLE) model mice ("BXSB" mice) and investigated the effect of induction of fully MHC-mismatched bone marrow mixed chimerism (E. Takeuchi & Y. Takeuchi, 2007).

3. Treatment of autoimmune glomerulonephritis in BXSB lupus mice

3.1 BM mixed chimerism can be induced in BXSB mice

BXSB mice spontaneously develop autoimmune disease with features similar to human SLE. The disease is associated with auto-antibodies to self-antigens (Ags) including double strand (ds) DNA, single strand (ss) DNA, anti-platelet antibodies (Abs) and anti-erythrocyte Abs, with accompanying splenomegaly and lymphadenopathy. Immune complex-mediated nephropathy is the hallmark disease associated with the BXSB genotype. Histopathological changes are evident by 10 weeks of age, leading to end-stage renal disease and 70% mortality by 40 weeks of age. We sought to determine whether the simple regimen described above was also effective for the induction of long-term mixed chimerism in BXSB mice. Twenty million normal bone marrow cells from MHC-matched (B6/GFP: H-2^b) or -mismatched (BALB/c: H-2^d) donors were injected with 2.0mg MR1 (i.p.) to seven-week old BXSB mice (H-2^b) that had received a nonlethal dose of 4Gy TBI one day prior to BMT. We increased the TBI dose for BXSB mice from 3 to 4Gy because BXSB mice are more resistant to engraftment than normal recipients are. This regimen allowed the induction of multi-lineage mixed chimerism in 70-90% of host BXSB mice.

As shown in Table 1 and Fig.2, long-term stable chimerism was observed in MHC-mismatched chimeric mice. No clinical signs of GVHD were seen during the observation period.

	CD4 Tcell	CD8 Tcell	B cell	macrophage
20wks after BMT	73.9 ± 11.3	46.9 ± 10.8	51.0 ± 11.8	94.9 ± 4.02
40wks after BMT	78.1 ± 13.1	56.1 ± 17.6	68.6 ± 14.4	85.2 ± 7.75

Table 1. The percentage of donor cells among PBL of chimeric mice. (n=9)

To confirm the establishment of donor-specific tolerance, chimeric BXSB mice also received skin grafts from a BM donor and a third party (C3H/HeN, H-2^k) one day after BMT. All chimeric BXSB mice accepted the donor skin, but rejected the third-party skin grafts within 20 days (Fig.3), indicating that chimeric BXSB mice acquired donor-specific tolerance without immune-deficiencies (E. Takeuchi, 2011).

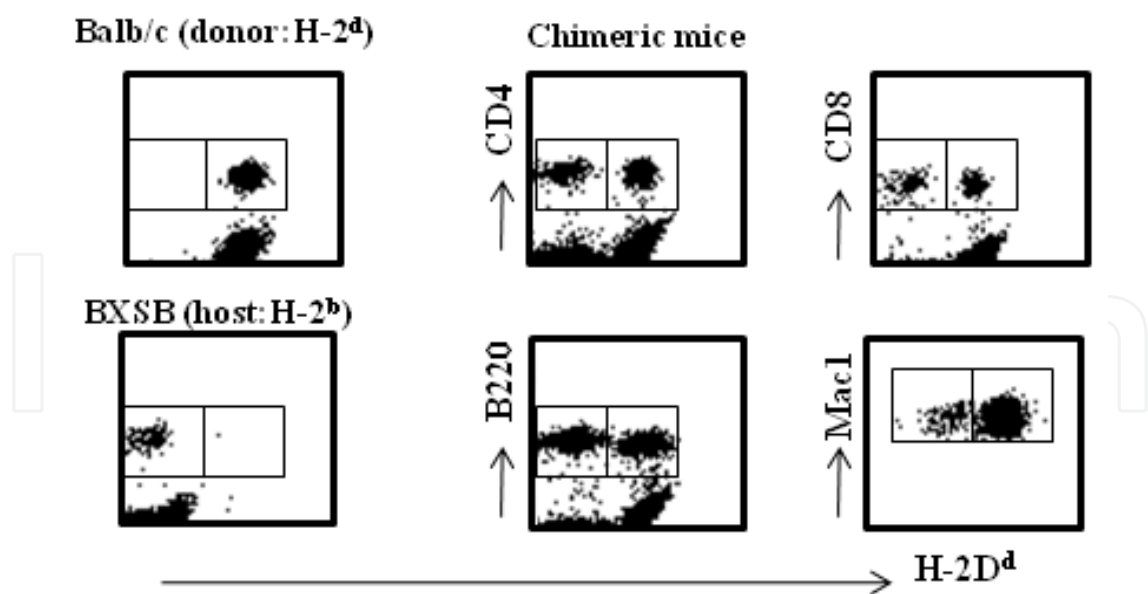


Fig. 2. An example of chimerism in peripheral blood lymphocytes (PBL). The percentage of BALB/c (H-2D^d) donor cells present among PBL of various lineages was analyzed through two-color FACS. These data were obtained 24 weeks after BMT.

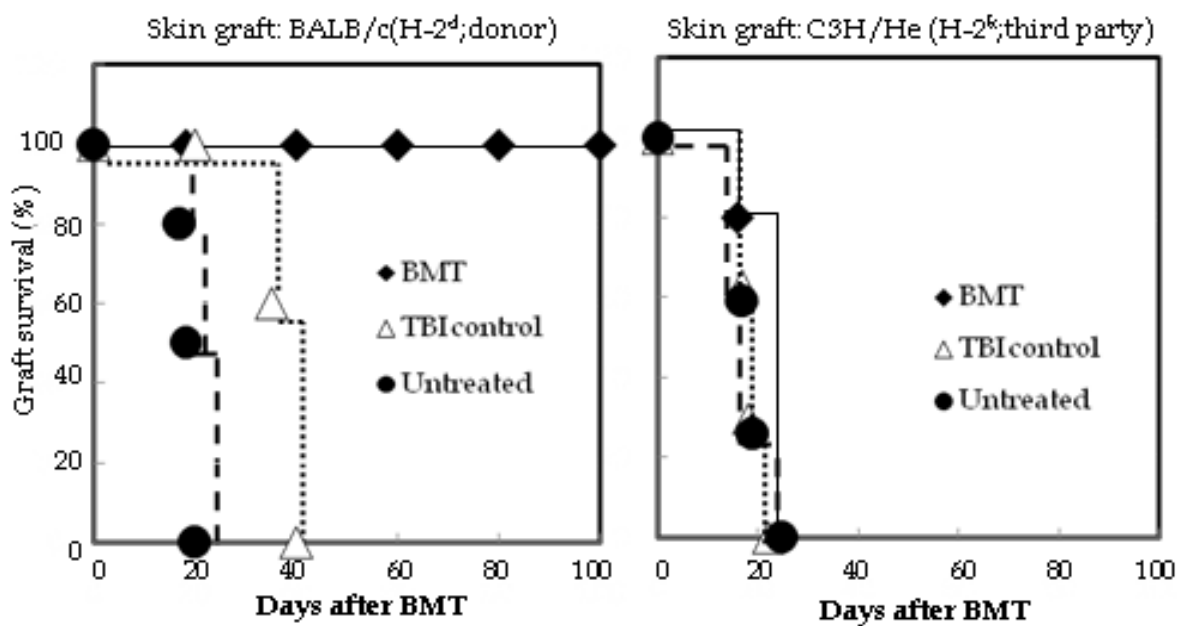


Fig. 3. Donor-specific skin graft tolerance. Donor and third-party skin was grafted 1 day post-BMT. Chimeric mice receiving MHC-mismatched BALB/c BM (◆BMT: n=9) accepted BALB/c skin grafts permanently, while third-party skin was rejected. Mice treated with TBI and anti-CD40L Ab (△: n=5) and mice receiving no treatment (●:n=5) rejected both donor and third-party skin.

These results indicate that, with regard to reciprocal tolerant between donor and host, T cells in stable mixed chimeric mice do not reject additional tissue grafts transplanted from the same donor. In lupus patients who suffer from renal disorders, and who are treated by means of kidney transplantation, the induction of specific immunologic tolerance to donor

antigens would prevent both chronic graft rejection and the side effects associated with chronic, nonspecific immunosuppressive therapy.

3.2 Induction of mixed chimerism suppressed lupus nephritis in BXSB mice

Even when transplanted kidneys are engrafted stably, when pre-existing lupus goes untreated, renal disorder will eventually recur. It is also known, however, that the induction of mixed chimerism reverses the autoimmune state. To evaluate the effect of inducing MHC-matched or MHC-mismatched mixed chimerism, individual kidneys were harvested from experimental mice and tissue sections were stained with periodic acid-Schiff (PAS) for histopathologic examination. None of the donor mouse strains were prone to autoimmune disease (Fig.4A). In both the fully MHC-matched and the fully MHC-mismatched chimeric mice groups, lupus glomerulonephritis was significantly ameliorated compared with that in untreated BXSB mice, as revealed by pathological analysis conducted more than 40 weeks after BMT (Figs.4D and E).

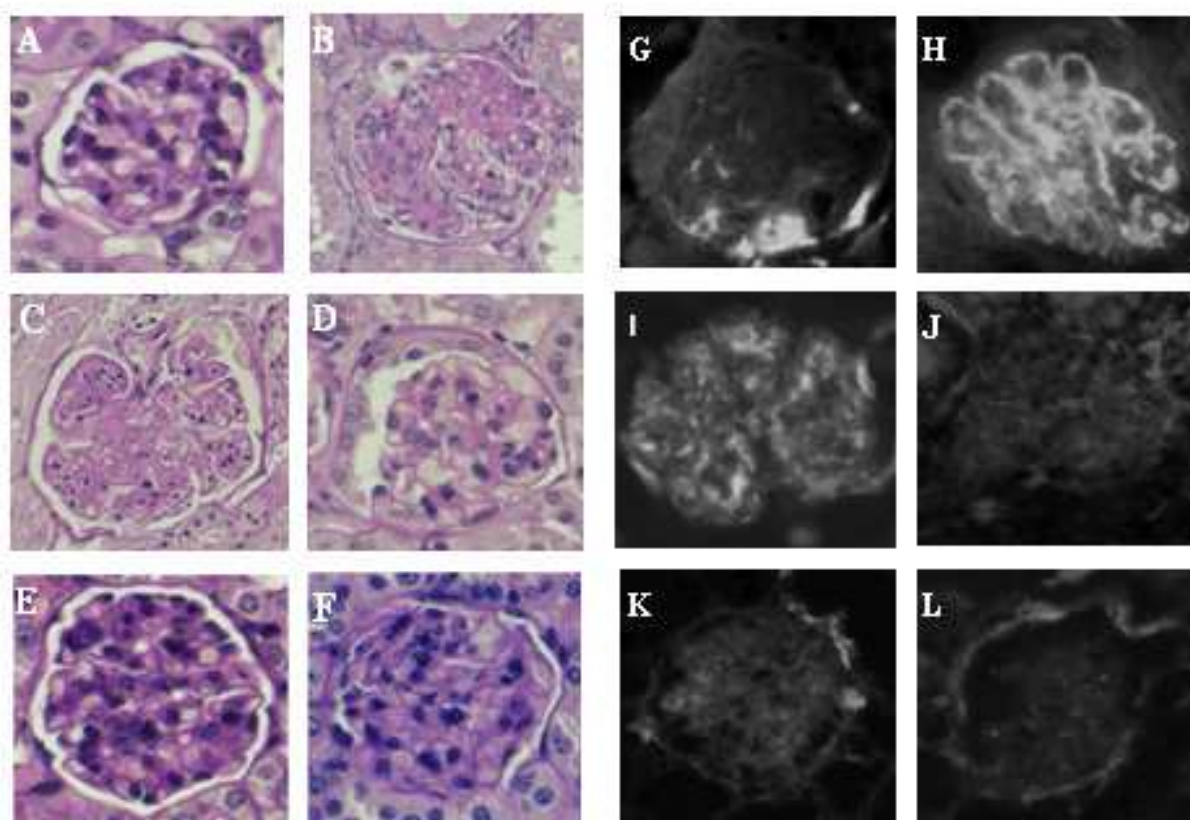


Fig. 4. Kidney sections stained with PAS (left panel) and IF with anti-mouse complement C3 (right panel). Kidneys were harvested from normal control mice or 47-50-week-old (40-43 weeks after BMT) BXSB mice with or without the indicated treatments. C57BL/6 control mice (A and G), untreated BXSB mice (B and H), irradiated BXSB mice (C and I), BALB/c BMT chimeric mice (D and J), GFP/B6 BMT chimeric mice (E and K), BALB/cxGFP/B6 F1 BMT chimeric mice (F and L).

Both untreated (Fig.4B) and irradiated BXSB mice (Fig.4C) exhibited severe glomerulonephritis with PAS-positive deposits. BXSB mice that had received TBI+MR1

exhibited histopathology similar to BXSB mice that had received only TBI (data not shown). For semiquantitative histologic analyses, more than 30 glomeruli from each kidney section were examined. Glomerulonephritis was scored on a scale of 0-4, based on the intensity and extent of histopathological changes [0; no glomerular lesions. 1; minimal thickening of the mesangium. 2; noticeable increase in both mesangial and glomerular capillary cellularity. 3; same as 2 with the addition of superimposed inflammatory exudates and capsular adhesions. 4; obliteration of the glomerular architecture (>70% of glomeruli)]. Mean renal scores of both MHC-matched and -mismatched chimeric mice were significantly better than those of untreated or irradiated control BXSB mice (BALB/c BMT: 0.97 ± 0.74 , GFP/B6 BMT: 0.07 ± 0.26 vs. untreated BXSB: 2.96 ± 0.72 , TBI: 1.85 ± 0.77 , TBI+MR1: 2.47 ± 3.4 , >30 glomeruli from each kidney section in three mice of each group were evaluated. $p < 0.05$). We also evaluated immune-complex mediated glomerulonephritis through immunofluorescence (IF) staining with anti-mouse complement C3 in 30 glomeruli per renal section (Fig.4. right panels). Untreated mice and irradiated mice exhibited the peripheral loop pattern (Figs. 4H and I), while almost all glomeruli in the sections from chimeric mice showed negative staining (Figs. 4J and K). Because the cause of death in BXSB mice is most often renal failure, the improvement of their glomerulonephritis may have contributed to the prolongation of their life-spans. These results indicate that the induction of mixed chimerism significantly inhibited the development of lupus-like disease.

It should be noted, however, that the induction of mixed chimerism in BXSB mice could not completely eliminate auto-reactive host lymphocytes because our regimen retains certain stem cells and lymphocytes belonging to the host. This naturally leads to the question of how donor cells reverse the host autoimmune state, which is discussed in the next section.

4. How does BM chimerism reverse the autoimmune state?

4.1 Hypothesis

We and several other groups have shown that the induction of bone marrow mixed chimerism is an effective treatment for and/or means of prevention against the development of autoimmune disease. Previous studies have debated the mechanisms that may be responsible for the reversal of the autoimmune state in BM chimerism, but the mechanism of the exclusion of self-reactive lymphocytes has not yet been conclusively identified. Preceding studies have argued about the mechanisms underlying the reversal of the autoimmune state in BM chimerism. In several studies which reversed destructive autoimmune type I diabetes (NOD mice with induced mixed chimerism), the suppression of autoimmune disease was attributed to reciprocal clonal deletion or to anergy induction of T lymphocytes of recipient and donor origin (Mathieu et al., 1997. Nikolic et al., 2004). Other mechanisms have also been proposed, including induction of peripheral anergy, a change in the Th1/Th2 profile, correction of abnormal secretion of cytokines and positive selection of regulatory T cells in the thymus. Among proposed hypotheses, we have focused on the role of cognate TCR/MHC interactions in the pathogenesis of autoimmune disease in BXSB mice (E. Takeuchi et al., 2011)

4.2 The induction of MHC-mismatched chimerism does not suppress anti-DNA Abs

During the development of their lupus-like autoimmune disease, BXSB mice are known to produce auto-antibodies to self-antigens including dsDNA. We measured serum anti-

dsDNA antibody (anti-DNA Ab) levels by means of ELISA to evaluate whether auto-reactive Abs were eliminated by the induction of mixed chimerism. Actually, the anti-DNA Ab levels in both MHC-matched and MHC-mismatched chimeric mice were lower than those in untreated or irradiated BXSB mice. Meanwhile, anti-DNA Ab levels in fully MHC-matched mixed chimeric mice (GFP/B6 BMT) were not statistically different from those in normal control B6 mice, but those in fully MHC-mismatched mixed chimeric mice (BALB/c BMT) were significantly higher than those in normal controls. This tendency was even more pronounced when anti-DNA IgM levels in the above groups were compared. There were no significant differences in anti-DNA IgM levels between MHC-mismatched chimeric mice and untreated or TBI+MR1 mice, even though total anti-DNA levels were much lower in chimeric mice than in other groups.

These data indicated that anti-DNA Ab producing cells were still present in the BXSB chimeric mice, though they stopped switching iso-types from IgM to IgG in MHC-mismatched chimera. Only in MHC-matched chimeric mice could the expansion of anti-DNA Ab production be suppressed down to a normal level; the induction of fully MHC-mismatched chimerism did not completely suppress or eliminate anti-DNA-producing B cells.

4.3 Selective suppression of auto-reactive antibodies in chimeric mice

In order to distinguish the contributions of donor-type and host-type B cells to anti-dsDNA antibody production, we determined IgM allotypes [IgMa: BALB/c (donor), IgMb: BXSB (host)] in the serum of fully MHC-mismatched (BALB/c→BXSB) chimeric mice 20 weeks after BMT. At this point, the percentage of allogeneic donor B cells in the chimeric mice was 51.0±11.8%, indicating that allogeneic donor and host B cells had contributed equally to the immune response. Surprisingly, however, we found that the majority of the anti-DNA IgM was IgMa (allogeneic donor-type), whereas IgMb (host-type) anti-DNA antibody production was suppressed (Fig. 5A).

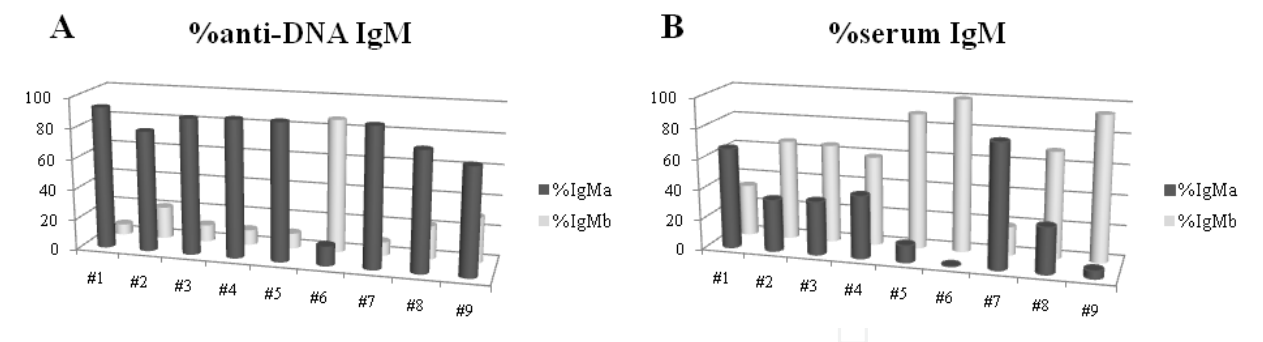


Fig. 5. The ratio of host-type IgM to donor-type IgM. (A) Serum anti-DNA IgM in BALB/c BMT BXSB was measured in each sample by means of ELISA. The percentages of anti-DNA IgMa (donor-type IgM: dark bar) and IgMb (host-type IgM: light bar) add up to the total anti-DNA IgM. (B) Total IgM in each BALB/c BM- transplanted BXSB mouse was measured individually. The percentages of serum IgMa (donor-type IgM: dark bar) and IgMb (host-type IgM: light bar) add up to the total IgM.

The majority of the total serum IgM, on the other hand, was host-type IgMb (Fig. 5B), suggesting that the production of “normal” serum Ig is dependent on host MHC-restricted T cells. Total serum IgM levels in fully MHC-mismatched chimeric mice were not significantly

different from those in other groups (data not shown). These results indicated that normal B cells derived from donor BALB/c mice, rather than genetically lupus-prone host-type B cells, were responsible for anti-DNA antibody production in these chimeric BXSB mice. Thus the regulation of auto-antibody production appears to be under MHC-restriction of the host type. Additionally, to confirm which set of B cells (donor-type or host-type) could react with foreign antigens, sheep red blood cells (SRBC) were administered intraperitoneally to five BALB/c chimeric BXSB mice. Three days after immunization, serum anti-SRBC Ab was detected through flow cytometry. All chimeric mice produced antibodies that were reactive with SRBC. As expected, almost all of the detected anti-SRBC Ab was IgM β (host-type), not IgM α (allogeneic donor-type) (data not shown). In mixed chimeras, all peripheral T cells are supposed to be restricted to host MHC, because of positive selection in the host thymus. T cells in fully MHC-mismatched chimeric mice should therefore be capable of cognate interaction with host-type B cells but not with donor-type B cells. Accordingly, only host-type B cells were activated by antigens through cognate interaction with helper T cells. Donor-type B cells remained “silent” because they could not interact properly with T cells. Interestingly, however, our results indicated the possibility that not only “proper” activation against foreign antigens, but also suppression of auto-reactive antibody production were regulated through TCR/MHC cognate interactions.

4.4 Do T cells survey auto-reactive antibody production?

Based on these data, we drew the following conclusions: 1. Allogeneic BM chimerism ameliorates autoimmune disease, but fully MHC-mismatched chimerism fails to suppress the production of anti-DNA antibodies. 2. In MHC-mismatched mixed chimeras, anti-DNA antibodies are produced by donor-type B cells rather than host-type B cells. 3. In MHC-mismatched chimera, TCRs are restricted to host-type MHC. Accordingly, T cells can recognize only host B cells but not donor B cells. To tie theses conclusions together, we propose a possible T cell surveillance system of mixed chimerism, as depicted in Fig.6.

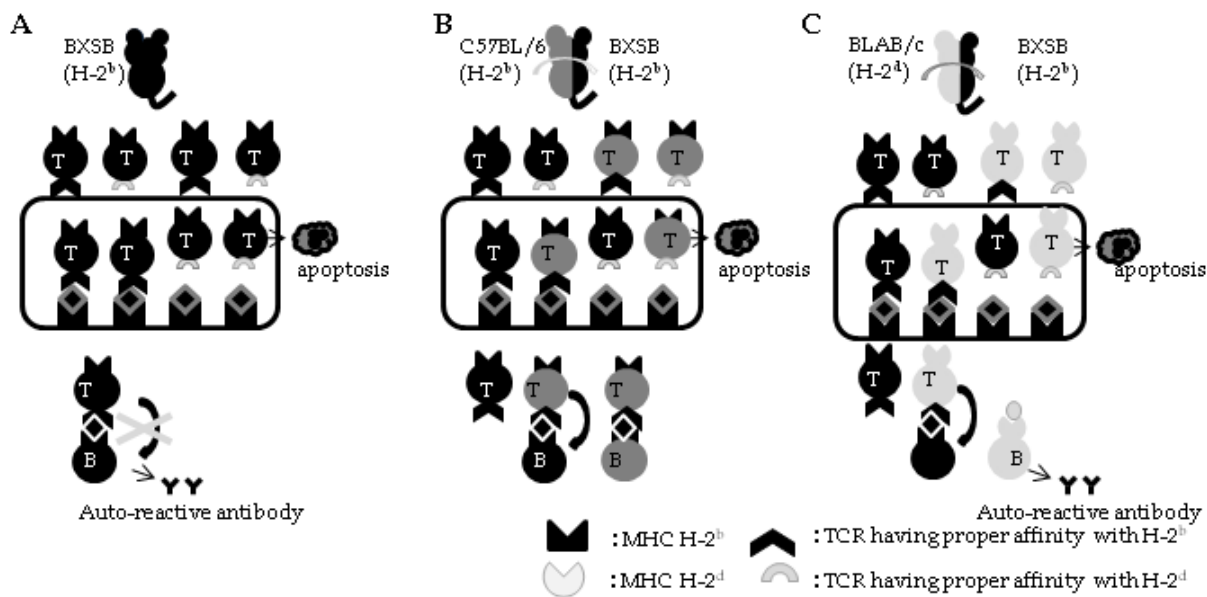


Fig. 6. The proposed of T cell surveillance model.

T cell precursors derived from BM differentiate to mature T cells in the host thymus. Through a process known as positive selection, all T cell populations can interact with self-MHC with proper affinity. Since these processes are performed by MHC molecules expressed on thymic epithelial cells, the TCR repertoire in mixed chimera is restricted to host MHC. We and others speculate that under genetically normal conditions, T cell-mediated trimming of autoantibody production may occur through cognate interactions between TCR and MHC+peptide presented on B cells (Rathmell et al., 1995, Shinohara et al., 1997). In the case of the BXSB mouse, it is known that T cells have certain defects which might play an important role in the pathogenesis of autoimmunity (Wofsy, 1986). We also speculate that the pivotal defect of BXSB may be a genetic defect in this surveillance function of T cells (Fig.6A). In the case of fully MHC-matched chimerism, T cells derived from donor BM can take this place of defective host T cells. Auto-reactive B cells derived from both donor and host BM can be regulated or trimmed by donor T cells through TCR/MHC interactions (Fig.6B). In MHC-mismatched chimerism, immature T cells are positively selected on the basis of their weak reactivity with self-peptides presented exclusively on H-2^b MHC molecules, since thymic epithelial cells express only host MHC. Therefore, in the periphery, all T cells recognize antigens presented by APCs on H-2^b MHC molecules (BXSB: host type MHC). Yet, B cells expressing H-2^d MHC (BALB/c: donor type MHC) are still generated from donor BM stem cells as this process is genetically determined. T cells developing in the BXSB thymus should be incapable of cognate interactions with these “wrong” MHC molecules expressed on donor B cells. We speculate that the failure of cognate interaction with T cells might be the reason why auto-reactive antibody levels rose in MHC fully-mismatched chimeric mice (Fig.6C).

The present study did not address the question of how anti-self B cells were initially triggered. We also induced fully MHC-mismatched BM chimerism in normal B6 mice (BALB/c→B6) as opposed to lupus BXSB mice. The anti-DNA Ab levels seen in BALB/c→B6 chimeric mice were slightly higher than those in normal B6 mice, but, much lower than those seen in BALB/c→BXSB chimeric mice (data not shown). This means that the induction of fully MHC-mismatched chimerism in normal mice may carry a risk of autoantibody production, though uncertain factors in host BXSB mice drives a non-physiological priming of B cells.

4.5 BMT from haplo-identical donor effectively suppressed auto-antibody production

If there is indeed a host-type MHC restriction in the suppression of auto-reactive antibody production, BMT from a donor with partially identical MHC that is sufficient to maintain cognate interaction should be equally as effective as BMT from a fully MHC-matched GFP/B6 mouse. To test this hypothesis, BM cells taken from BALB/c (H-2^d) × GFP/B6 (H-2^b) F1 mice with haplo-identical MHC (H-2^{b/d}) were transplanted to BXSB (H-2^b) mice. In this case, all B cells, even those differentiated from donor BM, contained at least one H-2^b allele. As shown in Figs.4F and L, lupus glomerulonephritis in F1 chimeric mice was alleviated to a degree comparable to that seen in MHC-matched GFP/B6 chimeric mice. Serum anti-DNA Ab in F1 chimeric mouse group was decreased to level comparable to that seen in fully MHC-matched GFP/B6 chimeric mice (data not shown).

The survival rates in both the GFP/B6 chimeric mouse group and the F1 chimeric mouse group, which were higher than that in the BALB/c chimeric mouse group, indicated that BMT from F1 mice is also effective as a treatment for lupus-like disease in BXSB mice (B6

BMT: 100%, F1 BMT: 80%, vs BALB/c BMT: 70%, TBI+MR1: 60%, TBI: 20%, untreated: 20% survival, 50weeks after BMT). These results suggest that the maintenance of TCR/MHC cognate interaction with all B cells is important in regulating auto-reactive Ab production, and that reconstitution of the T cell surveillance system may reverse the autoimmune state of lupus-like disease in the BXSB mouse. Moreover, our results indicate one possibility for clinical application: BMT between parent and child, both parent→child and child→parent, may be able to reverse the autoimmune state of SLE effectively.

5. Clinical application and unknown risks of mixed chimerism

This paper has demonstrated that the maintenance of TCR/MHC interaction with all B cells is important in regulating auto-reactive Ab production. However, we and several other groups have reported that the induction of fully MHC-mismatched chimerism is certainly effective as a treatment for autoimmunity. How does the induction of fully MHC-mismatched mixed chimerism suppress autoimmune disease? One answer to this question is demonstrated by the results of an immunohistochemical experiment in which we stained for several isotypes of immunoglobulin.

As depicted in Table 2, linear staining patterns with IgG and/or IgM were definitely observed on the glomeruli of kidney sections taken from MHC-mismatched chimeric mice; the same sections were negative for C3 depositions, however. Linear staining with both IgG and C3 was observed on the glomeruli taken from untreated mice.

		C3	IgG				IgM
treatment				IgG1	IgG2a	IgG3	
Untreated	1	+	-	-	-	-	-
	2	+	M	-	-	M	M
	3	+	-	-	M	-	M
	4	+	+	-	-	+	+
	5	+	+	+	M	-	+
BALB/c BMT	1	M	+	-	-	-	+
	2	-	-	-	-	-	+
	3	-	-	-	-	-	
	4	-	+	-	-	-	+
	5	-	-	-	-	-	+

+: linear staining, -: negative staining M: mesangeal staining

Table 2. Immunofluorescence staining with several isotype Ig of glomeruli.

This indicates that antibodies deposited in fully MHC-mismatched chimeric mice, mainly IgM, did not activate the complements effectively. As a result, lupus glomerulonephritis is milder in chimeric mice than in untreated mice. We presume that class-switching from IgM to other isotypes does not occur on donor anti-dsDNA IgM-producing B cells, since TCR/MHC cognate interactions were disrupted.

Given that T cells could neither activate nor suppress B cells, B cells are expected to be inactive or “silent”. In fact, we have confirmed that, when fully MHC-mismatched

chimeric mice are immunized with foreign antigen (sheep red blood cells, SRBC), antigen-specific antibodies (anti-SRBC IgM) are mainly produced by host B cells (as mentioned in 4.3). In rare cases, however, especially when immunization with the same antigen is repeated several times, donor B cells accidentally respond and produce specific antibodies. A T cell that is specific for one peptide on an MHC molecule may cross-react with peptides presented by other allogeneic MHC molecules. By these accidental interactions, donor B cells are activated and start to produce specific antibodies. During activation and proliferation, B cells undergo variable-region somatic hypermutation and change their antigen affinity, resulting in the generation of variant immunoglobulins, some of which are thought to bind to the original foreign antigen with higher affinity. However, the potential disadvantage of this process is that some of the antibodies could be auto-reactive.

To test this hypothesis, we induced an auto-cross-reactive antibody (Ab) by introducing a foreign antigen with a homolog to an auto-antigen into a BXSB lupus mouse strain of mixed chimerism with several combinations of donor BM. The titer of auto-cross reactive foreign Ab plateaued at low levels in normal mice and MHC-matched/haplo-identical chimeric mice, but rose higher in BXSB and fully MHC-mismatched chimeric mice (unpublished data). These results indicate that the induction of fully MHC-mismatched chimerism may carry a risk of secondary auto-antibody production.

Under normal conditions, these auto-reactive B cells may be anergic. In lupus patients, however, non-physiological factors may prime auto-reactive B cells. For example, it has been reported that circulating B cell activating factor (BAFF) is elevated in the serum of human patients with lupus, and that the overexpression of BAFF in mice promotes TLR-induced production of auto-antibodies through a T cell independent process (Groom et al., 2007). Under autoimmune conditions, normal B cell may have the potential to run off the rails. The maintenance of TCR/MHC interaction may be a “rein” by which immune-response is controlled in chimeras.

Because several mechanisms have been suggested as drivers of autoimmune disease, further study is necessary to identify each mechanism’s role. Nevertheless, our results showing the specific suppression of auto-reactive antibody production suggest the existence of a surveillance system that trims auto-reactive B cells after priming. The reconstitution of this surveillance system through the induction of BM mixed chimerism can be an effective treatment for lupus disease. Moreover, the induction of BM mixed chimerism with haplo-identical donor BM, which maintains cognate T/B interaction with both donor and host cells, can be equally effective as fully MHC-matched donor BM. These results may support the clinical application of BMT as a treatment for lupus disease.

6. Conclusions

Induction of BM mixed chimerism can be useful for treatment of refractory lupus glomerulonephritis. Elucidation the mechanism through which mixed chimerism reverses the autoimmune state is necessary for clinical application.

In this paper, we suggested the existence of T cell surveillance system through TCR/MHC interaction. Through TCR/MHC interaction, T cell-mediated trimming of auto-antibody production may occur under normal condition. The induction of bone marrow mixed chimerism may reverse the auto-immune state through reconstruction of the T cell

surveillance system and the maintenance of TCR/MHC interaction with all B cells is important in regulating auto-antibody production.

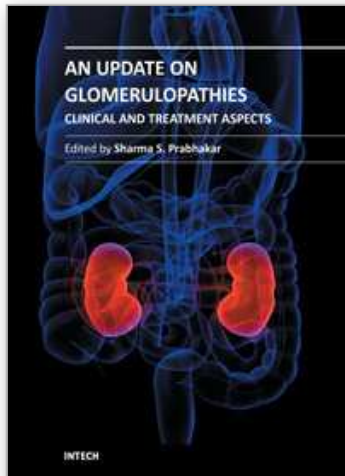
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An Update on Glomerulopathies - Clinical and Treatment Aspects

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An Update on Glomerulopathies - Clinical and Treatment Aspects is a systemic overview of recent advances in clinical aspects and therapeutic options in major syndromes of glomerular pathology. The book contains twenty four chapters divided conveniently into five sections. The first section deals with primary glomerulopathies, and the second section is devoted to glomerulopathies complicating infectious conditions. The third section deals with systemic autoimmune disorders and vasculitides which constitute major causes of glomerular disease and often renal failure. The fourth section includes chapters discussing the glomerular involvement in some major metabolic and systemic conditions. The final section has chapters which relate to some general aspects of glomerular diseases. This book will form an excellent reference tool for practicing and academic nephrology community.

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