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The Influence of Immunosuppressive Agents on the Risk of De Novo Donor-Specific HLA Antibody Production in Solid Organ Transplant Recipients

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Production of de novo donor-specific antibodies (dnDSA) is a major risk factor for acute and chronic antibody-mediated rejection and graft loss after all solid organ transplantation. In this article, we review the data available on the risk of individual immunosuppressive agents and their ability to prevent dnDSA production. Induction therapy with rabbit antithymocyte globulin may achieve a short-term decrease in dnDSA production in moderately sensitized patients. Rituximab induction may be beneficial in sensitized patients, and in abrogating rebound antibody response in patients undergoing desensitization or treatment for antibody-mediated rejection. Use of bortezomib for induction therapy in at-risk patients is of interest, but the benefits are unproven. In maintenance regimens, nonadherent and previously sensitized patients are not suitable for aggressive weaning protocols, particularly early calcineurin inhibitor withdrawal without lymphocyte-depleting induction. Early conversion to mammalian target of rapamycin inhibitor monotherapy has been reported to increase the risk of dnDSA formation, but a combination of mammalian target of rapamycin inhibitor and reduced-exposure calcineurin inhibitor does not appear to alter the risk. Early steroid therapy withdrawal in standard-risk patients after induction has no known dnDSA penalty. The available data do not demonstrate a consistent effect of mycophenolic acid on dnDSA production. Risk minimization for dnDSA requires monitoring of adherence, appropriate risk stratification, risk-based immunosuppression intensity, and prospective DSA surveillance.

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De novo formation of donor-specific antibodies (DSA) directed against HLA has been identified as a major risk factor for antibody-mediated rejection (AMR).[1] Production of de novo DSA (dnDSA) is associated with an increased risk of graft failure in all types of solid organ transplantation: kidney,[2-4] kidney-pancreas,[5] liver,[6] simultaneous liver-kidney,[7] small bowel,[8] heart,[9,10] lung,[11,12] and pancreatic islet[13] transplantation. In the medium- to long-term, although late acute AMR can occur, chronic AMR is more common and represents the most common cause of late allograft dysfunction.[6,14,15] Patients with HLA class II or both class I + II DSA are at the greatest risk for chronic AMR[16] with anti-DQ dnDSA being the predominant specificity in kidney,[17-19] liver,[6] heart,[20] and lung[21] transplant patients. This occurs more frequently in nonadherent patients.[22,23] Clinical presentation varies between organs and includes acute and chronic graft dysfunction arising from microvascular injury leading to progressive fibrosis and loss of function.[9,10] Chronic AMR in kidney transplant patients may manifest as subclinical or clinically evident proteinuria with a slow, progressive loss of graft function over several years,[24,25]

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characterized by histopathologic changes, with or without C4d staining, and the presence of DSA in serum.[26] In kidney transplantation, it is estimated that graft loss may occur in 15% to 20% of cases within 1 year of AMR being diagnosed.[27] Chronic AMR is associated with acute hemodynamic compromise, accelerated transplant coronary artery disease and mortality after heart transplantation,[15,28] and graft injury and fibrosis in liver transplants.[29,30] The dnDSA development in lung transplant recipients is a major risk for progression to bronchiolitis obliterans syndrome and greater severity of and death related to bronchiolitis obliterans syndrome.[14,31,32]

Research into the presence and clinical impact of dnDSA received a major impetus after the development of solid-phase assays, which improved the sensitivity of detection and characterization of HLA antibodies compared to previous complement-dependent cytotoxicity assays.[33,34] The near-universal adoption of single-antigen beads for specificity testing, moreover, has made it possible to differentiate between dnDSA and non-DSA more accurately.[33] Current techniques also permit investigation of the biological activity and mechanisms of antibody injury. For instance, complement-binding (C1q) dnDSA appears to show a stronger relationship with graft loss than non-C1q–binding antibodies.[1,35,36] Considerable challenges persist, however, including intermanufacturer and lot-to-lot variation, a lack of standardization in cutoff points to define a positive test, and a degree of intralaboratory and interlaboratory variabilities.[34,37] Variability between laboratories using the solid-phase antigen bead assay with Luminex technology can be reduced by standardizing the test protocol and using identical reagents.[34] The DSA measurement using this technique can assess strength, effector function (via analysis of complement fixing properties, although false positive or negative results are possible), and immunoglobulin G subclasses. Furthermore, xenoantibodies, such as rabbit antithymocyte globulin (rATG) and monoclonal antibodies, such as rituximab, may interfere with some antibody detection methods, such as complement-dependent cytotoxicity and flow cytometric crossmatch[37-40] but not with solid phase antigen bead assays. Thus, comparison of dnDSA results between studies can be confounded by potential differences in the immunosuppression administered or in the timing and type of monitoring techniques used during follow-up.

Because dnDSA development has been convincingly associated with inferior outcomes,[4,41] it is imperative to avoid this undesirable alloimmune response, but simple overimmunosuppression carries significant risks, and may still be insufficient to control a robust antibody response. Therefore, it is essential to understand the risk factors for dnDSA formation and the relative effects that each immunosuppressive agent may have on prevention of dnDSA formation.

Toward the goal of risk-based personalized immunosuppression, this review evaluates the influence of induction and maintenance immunosuppression on the likelihood of dnDSA formation.

IMMUNOSUPPRESSION-INDEPENDENT RISK FACTORS FOR dnDSA PRODUCTION

Donor and recipient characteristics alter the risk of dnDSA formation. In particular, the degree of HLA matching is a major independent predictor of dnDSA formation.[6,41,42] Although only mismatching at HLA A, B, or DR loci has traditionally been evaluated, currently DQ mismatching appears consistently associated with the highest frequency of dnDSA.[6] Across all organs, including pediatric recipients, dnDSA are mostly directed against DQ antigens, in particular DQ β chains.[6,19,28,43] Other HLA loci, however, cannot be neglected. Wiebe and colleagues[41] observed HLA-DRβ1, but not HLA-DQ, to be an independent predictor for dnDSA in a series of 315 kidney transplant patients without preformed DSA, and a significant effect of HLA-DR matching has been described by other authors.[4] Future, more sophisticated, epitope mismatching analyses may prove useful in risk assessments of dnDSA development.[44-46]

Younger age (typically, <50 years) is consistently associated with increased risk of dnDSA development.[6,42,47-49] This may result from a more robust immune system or may simply be a consequence of greater nonadherence. African American race,[43] male sex,[48] the pre-existence of non-DSA HLA at the time of transplantation, and persistent BK virus infection[50] also appear to influence risk. Other important factors include sensitization events, such as retransplantation,[47,51] pregnancy,[52] and blood transfusions[51,53] or indicators of high immunological risk, such as previous acute rejection.[4,41,47,48,54,55]

In the absence of randomized controlled trials evaluating dnDSA development between different immunosuppressive therapies, this array of risk factors complicates comparisons between studies, particularly where multivariate analyses are not performed. They should, however, be taken into account when customizing immunosuppressive strategy. It is critical to also remember that dnDSA develops over time; numerically more patients become dnDSA positive as time posttransplant increases, which must be considered when comparing studies.

EFFECT OF INTENSITY OF IMMUNOSUPPRESSION

Nonadherence or Inadequate Immunosuppression

Underimmunosuppression is known to lower kidney allograft survival[56] and, intuitively, would be expected to increase the likelihood of dnDSA production. Two distinct scenarios heighten concern for dnDSA development: (1) nonadherence to the prescribed regimen and (2) underimmunosuppression as a result of weaning strategies. Recent studies of nonadherence to the immunosuppressive regimen[57,58] have shown nonadherence to affect at least a quarter of patients across all organs, and it increases over time after transplantation. Nonadherence is a well-established risk factor for dnDSA[23,41,59] and late acute AMR.[16,22] Data from small single-center series in adult[59,60] and pediatric[61] kidney transplants have described a high rate of nonadherence or prescribed reduction in immunosuppression in the majority of patients with dnDSA[59,61] or AMR.[60] In 1 report of 23 cases of AMR, 4 patients had documented nonadherence, whereas 16 patients had previously received a physician-directed reduction in immunosuppression.[60] Sellarés et al[23] prospectively followed up kidney transplant patients for a median of 31.4 months after indication allograft biopsy. Nonadherent patients at the time of biopsy were more likely to be DSA positive (77% versus 29% in adherent patients, $P < 0.001$), and more likely to progress to graft failure (32% versus 3%, $P = 0.0001$) than adherent patients. Similarly, Wiebe et al[41] studied 315 consecutive DSA-negative kidney transplant recipients, 15% of whom developed dnDSA within a median of 4.6 years. Nonadherence was significantly more frequent in those with dnDSA

(49% versus 8% in adherent individuals, $P < 0.001$), a finding confirmed in logistic regression analysis (odds ratio [OR] = 8.75, $P < 0.001$). In liver transplantation, compliance with the calcineurin inhibitor (CNI) regimen was the most influential factor associated with dnDSA formation.[6]

Also, some medications can be associated with dnDSA production, such as interferon in renal allograft recipients.[62] Although the risk of dnDSA has never been tested in liver allograft recipients of interferon therapy, plasma cell hepatitis is a known complication and one can speculate this to be associated with dnDSA formation.[63] Therefore, this may educate us about the role of interferon, either endogenous or exogenous in dnDSA formation.

Weaning of Immunosuppression

Calcineurin inhibitor and steroid-sparing strategies have been widely investigated in all types of solid organ transplantation. Graft injury from antibody-mediated damage may increase if CNI therapy is withdrawn or becomes subtherapeutic.[64] A complete understanding of this effect, however, has to date been hampered by the lack of DSA data collection in the majority of earlier randomized CNI-sparing trials, and the paucity of long-term data on dnDSA monitoring after CNI reduction or withdrawal in more recent studies. Steroid withdrawal or avoidance may not increase the risk of dnDSA if adequate immunosuppression is otherwise maintained.[65,66] In a 5-year longitudinal study of 37 kidney transplants randomized to steroid withdrawal at day 7 or to standard steroid therapy, all of whom received rATG induction, tacrolimus and mycophenolate mofetil (MMF), Delgado et al[65] found that only one patient in the standard-steroids group developed dnDSA, and none in the steroid-withdrawal arm.

IMMUNOLOGICAL EFFECTS OF IMMUNOSUPPRESSIVE AGENTS

Although B-cells and plasma cells produce antibodies, T-cell help is essential for the development of dnDSA. Effective T-cell suppression is therefore crucial to prevent dnDSA formation. Figure 1 shows a schematic overview of the key immunosuppressant agents and classes and their targets, including helper T-cells, each of which is discussed in more detail below.

Biological Therapies

Rabbit antithymocyte globulin targets peripheral T-lymphocytes, B-lymphocytes, natural killer cells, and plasma cells, and to a lesser extent monocytes and macrophages.[67] Administration of rATG at a cumulative dose of 6 mg/kg depletes T-cells for up to 12 months[68] and may also reduce B-cells.[69] The monoclonal antibody alemtuzumab depletes both T- and B-cells for up to a year,[69,70] and primate models suggest that depletion is more complete than that with rATG.[71] The IL-2 receptor antagonist (IL-2RA) agents block activated T-cells without affecting T-cell or B-cell numbers.

Rituximab, a chimeric anti-CD20 monoclonal antibody, inhibits development of memory T-cells and modulates the B-cell response by depleting memory B-cells.[72] Recent publications show that rituximab could be of benefit in the induction of sensitized patients, and in abrogating rebound antibody response in patients undergoing desensitization or treatment for AMR.[73,74] The proteasome inhibitor bortezomib profoundly inhibits activated B-cells and induces plasma cell apoptosis.[75] Both agents have been used to treat refractory AMR[27] and for desensitization in the scenario of preformed antibodies.[76,77]

Maintenance Therapies

The CNI agents cyclosporine (CsA) and tacrolimus suppress the humoral immune response by interfering with T-helper cell signaling[78] and are potent suppressors of antibody-mediated natural killer cell activation in vitro.[64] CNI agents also attenuate T-cell–dependent B-cell immune responses by reducing levels of stimulatory cytokine mRNA in activated T-cells.[78] Mycophenolic acid (MPA) inhibits both T- and B-cell proliferation (by blocking guanosine nucleotide production and preventing DNA synthesis[79]) and T-cell trafficking through the transcription of GMP-dependent cell adhesion molecules.[79,80] The mechanistic target of rapamycin (mTOR) inhibitors everolimus and sirolimus block growth factor–mediated proliferation of T-cells and interfere with T-helper cell signaling.[81-83] They also suppress B-cell proliferation, B-cell immunoglobulin production in the early phase of the B-cell immune reaction,[84] and B-cell activation[85] and differentiation[86,87] and inhibit intracellular signaling implicated in AMR-induced allograft damage.[22,85,88,89] In a study comparing the immunologic effects of sirolimus, CsA and tacrolimus in a porcine model of arterial transplantation, dnDSA formation by day 30 was suppressed only in the sirolimus group.[90] Clinically, memory and regulatory T-cell recovery[91,92] during immune reconstitution after rATG or alemtuzumab induction is greater in kidney transplant patients treated with an mTOR inhibitor compared to CNI therapy.[91-95] Experimental models suggest that mTOR inhibition reduced noncomplement-mediated vascular injury by DSA.[96]

Corticosteroids exert a multifaceted immunomodulatory effect, altering T-cell function and redistributing cell subsets.[97] The B-cell antibody production is suppressed indirectly by steroids, through various mechanisms arising from a modified effect of T-cell function on allogeneic B-cell activation.[97]

IMMUNOLOGICAL EFFECTS OF IMMUNOSUPPRESSIVE DRUGS: CLINICAL EVIDENCE

The following sections assess the available data for individual immunosuppressive agents on dnDSA formation. Interpretation of these data is hampered by differences in intensity of overall immunosuppression, a paucity of prospective clinical trials and an abundance of multidrug cocktails. Despite these shortcomings, several hypothesis-generating possibilities can be considered. We will discuss drug classes and therapies separately.

Biological Therapies

Antithymocyte Preparations and IL-2RA Monoclonal Antibodies

In kidney transplantation, randomized controlled trials of antithymocyte globulins and IL-2RA agents have not reported data on dnDSA rates, although they have demonstrated a significant reduction in acute rejection with T-cell depletion therapy.[98-100] Rabbit antithymocyte globulin is an established induction agent in transplantation, and a possible treatment for AMR.[27] The effect of rATG induction therapy on propensity to produce dnDSA has been assessed in some nonrandomized trials (Table 1). In a recent single-center analysis of 114 consecutive DSA-positive kidney transplant

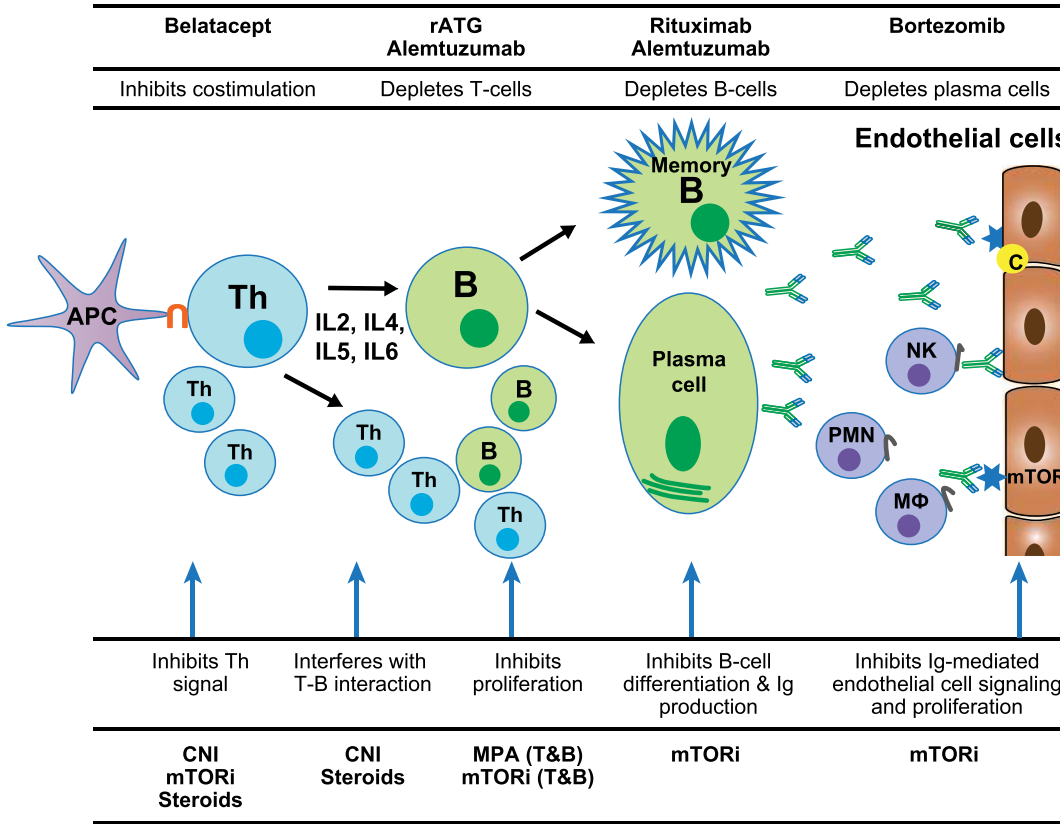

FIGURE 1. A schematic of the mode of action of key immunosuppressants. APC, antigen presenting cell; B, B-cell; C, complement; CNI, calcineurin inhibitor; Mφ, macrophage; mTORi, mammalian target of rapamycin inhibitor; NK, natural killer; PMN, polymorphonuclear cell; T, T-cell; Th, T-helper cell.

patients with a negative crossmatch who received rATG or basiliximab induction therapy, rATG was associated with a lower risk of dnDSA (hazard ratio [HR], 0.16; 95% confidence interval [95% CI], 0.04-0.50; $P = 0.003$) and AMR (HR, 0.16; 95% CI, 0.05-0.60; $P = 0.006$) in multivariate analysis.[101] Other retrospective studies observed no significant difference in the risk of dnDSA using IL-2RA versus rATG induction after kidney transplantation.[42,55] However, these studies were not designed to determine the role of induction immunosuppression on the incidence of dnDSA and rATG was used in higher immunological risk patients. Overall, rATG appears to achieve a short-term decrease in dnDSA production in moderately sensitized patients. Prospective randomized controlled trials are needed to assess the role of IL2-RA and antithymocyte globulins on the incidence of dnDSA.

Alemtuzumab

Randomized controlled trials of IL-2RA versus alemtuzumab have demonstrated a significant reduction in acute rejection with alemtuzumab[98,105] but did not provide information on the rate of dnDSA. When alemtuzumab induction was compared to basiliximab with low-dose rATG in a matched-cohort single-center study of kidney transplant patients, the incidence of dnDSA at 1 year after transplantation was higher in patients receiving alemtuzumab (50% [8/16]) than in the basiliximab/rATG control group (12.5% [4/32]; $P = 0.011$).[102] Other authors have observed a high rate of AMR with alemtuzumab after kidney-pancreas transplantation in patients receiving CNI maintenance therapy,[106] and an increased risk of AMR and dnDSA formation in alemtuzumab-treated kidney transplant patients receiving CNI-free immunosuppression.[69,107] Together, these data suggest that alemtuzumab as an induction agent may not be effective in preventing the early appearance of dnDSA, although new interventional randomized studies are needed to specifically address this question.

Rituximab

The chimeric monoclonal anti-CD20 antibody rituximab is an established option for treatment of refractory AMR,[27] although its effect on dnDSA prevention is less certain. In a nonrandomized trial of 320 unsensitized kidney transplant recipients, pretransplant rituximab did not influence dnDSA production.[108] In a prospective, double-blind trial of patients randomized to a single dose of rituximab or placebo induction, the incidences of AMR, biopsy-proven acute rejection, and dnDSA formation (rituximab, 3% [1/33]; placebo, 16% [6/38]) were similar at 3 years.[103] Several other centers have described their use of induction therapy with low-dose or single-dose rituximab in kidney,[109] liver,[110,111] and intestinal or multivisceral[112] transplantation, usually in combination with rATG, and have reported low rates of AMR (<4%) in patients with a positive crossmatch,[109,110] but data on dnDSA formation have not been reported. However, a randomized trial of rituximab versus IL-2RA induction in nonsensitized kidney transplant patients was terminated because of an excess of acute cellular rejection in the rituximab group (83% vs 14%, $P = 0.01$).[113] In contrast, in a recent retrospective analysis of 281 kidney transplant patients divided into four groups according to whether they had preexisting

TABLE 1.

Induction therapy: A comparison of the risk of dnDSA

Reference	Study design	N	Follow-Up	Induction regimen	Maintenance regimen	Univariate or multivariate* analysis dnDSA	P
rATG							
Brokhof et al, 2014[101]	Prospective Single center	114	3 y	rATG or BAS	TAC MMF Steroids	HR = 0.16* rATG vs BAS	0.003*
Huang et al, 2012[55]	Retrospective Cohort study Single center	145	1 y	rATG or BAS or None	CsA or TAC MPA Steroids	7.4% rATG 40% BAS 7.1% none	0.30
Alemtuzumab							
Todeschini et al, 2013[102]	Retrospective Matched control study Single center	48	2 y	ALEM or rATG/BAS	CsA or SIR MMF Steroids to day 7	57% ALEM 12.5% rATG/BAS	0.01
Rituximab							
Tydén et al, 2012[103]	Prospective Double-blind Multicenter	71	3 y	RITUX or Placebo	TAC MMF Steroids	3% RITUX 16% placebo	0.10
Bortezomib							
Ejaz et al, 2013[104]	Prospective Randomized 2 centers	40	1 y	rATG or rATG/RITUX or rATG/BORT or rATG/RIT/BORT	TAC MMF Steroids	30% rATG 30% rATG/RITUX 10% rATG/BORT 30% rATG/RITUX/ BORT	n/a

Asterisk indicates analyses were performed with multivariate analysis.

ALEM, alemtuzumab; BAS, basiliximab; BORT; bortezomib; n/a, not available; rATG, rabbit antithymocyte globulin; RITUX, rituximab; SIR, sirolimus; TAC, tacrolimus.

DSA and whether rituximab was administered, the rate of appearance of de novo HLA antibodies—and the incidence of chronic AMR—was lower in the groups that received rituximab treatment versus their untreated counterparts.[114] A Japanese report found that ABO-incompatible recipients either splenectomized or induced with low-dose rituximab developed dnDSA less frequently at 2 years than ABO-compatible recipients without either treatment (2.2%, 1.7%, and 18.1%, respectively).[109] Finally, preemptive treatment with rituximab and plasmapheresis was shown to increase the chance of clearing early dnDSA in a retrospective study of lung transplant recipients, although this did not appear to be associated with a clinical benefit.[115] Although there is adequate clinical evidence to support rituximab therapy for the treatment of AMR and possibly prevention of dnDSA, more randomized studies are needed to determine how and in whom it should be used.

Bortezomib

Off-label use of bortezomib, a first-in-class proteasome inhibitor, has yielded AMR treatment results.[116-121] This has prompted its use as part of induction regimens or after desensitization to target B-cells and plasma cells in combination with rATG.[104,122] In a randomized pilot study, Ejaz and colleagues[104] treated 40 kidney transplant patients at high immunological risk with rATG alone, rATG/rituximab, rATG/bortezomib, or rATG/rituximab/bortezomib. In total, 10 of 40 patients (25%) developed dnDSA within 1 year. Circulating dnDSA had cleared by 1 year in the rATG and rATG/bortezomib group, but not in the rituximab-treated cohorts. The incidence of AMR, however, was not significantly different between groups. In a study of 18 patients undergoing clonal depletion with donor-specific transfusion followed by treatment with bortezomib, rATG, rituximab, and steroids, 4 patients developed dnDSA, whereas 4 could be weaned off immunosuppression.[123] Similar results were reported in a small series of pediatric heart transplant cases, where bortezomib has been associated with a marked reduction in dnDSA and resolution of AMR.[124] In summary, the role of proteasome inhibitors in the prevention of dnDSA, while promising, remains to be determined in larger prospective studies.

Belatacept

The costimulation blocker belatacept prevents T-cell activation.[125] In the phase 3 registration trial (BENEFIT), AMR was avoided in all treatment arms, but acute cellular rejection was more frequent and more severe in the intensive belatacept treatment group compared to the CsA treatment group.[126] Similarly, no AMR occurred in the BENEFIT-EXT study of belatacept therapy in recipients of expanded criteria donors.[127] Numerically lower DSA rates at 1 year,[126] 2 years,[128] and 3 years[129] were seen in the BENEFIT patients treated with belatacept compared to those treated with CsA. However, no statistical comparison was performed, and preformed DSA were not distinguished from dnDSA. Therefore, belatacept may exert a protective effect on dnDSA formation, but prospective granular data are still needed.

Calcineurin Inhibitors

Calcineurin inhibitor administration, especially early after transplant, is likely protective against dnDSA formation (Table 2). In 244 consecutive kidney and kidney-pancreas

TABLE 2.

Calcineurin inhibitor maintenance therapy: A comparison of the risk of dnDSA

Reference	Study design	Organ type	N	Follow-up	maintenance regimen	Univariate or multivariate* analysis		Use of Therapy according to presence/absence of dnDSA
						dnDSA	*P*	
Del Bello et al, 2014[47]	Retrospective Cohort study Single center	Liver	232	Median 36.5 mo	Various	n/a	n/a	CNI therapy was used[a] in 81% and 80% of patients with or without dnDSA, respectively
Kaneku et al, 2013[6]	Retrospective Cohort study Single center	Liver	749	1 y	CsA or TAC ± MMF/AZA ± Sirolimus ± Steroids	OR = 2.5 CsA vs TAC*	0.004*	
Cooper et al, 2011[42]	Retrospective Cohort study Single center	Kidney or kidney-pancreas	244	2 y	Various	n/a	n/a	TAC was used[a] in 77% of patients with dnDSA and 90% of patients with no dnDSA ($P = 0.009$)
Huang et al, 2012[55]	Retrospective Cohort study Single center	Kidney	145	1 y	CsA or TAC MPA Steroids	22% CsA 4% TAC	0.02	
Lachmann et al, 2006[130]	Prospective Cohort study Single center	Kidney	1043	>6 mo to 4 y	CsA or TAC ± MMF/AZA ± Steroids	35% CsA[b] 20% TAC[b]	0.05	
Hourmant et al, 2005[4]	Prospective Cohort study Single center	Kidney	1229	5 y	Various	—	—	CNI was used[a] in 93% and 95% of patients with or without dnDSA, respectively (n.s.)

Asterisk indicates analyses were performed with multivariate analysis.
[a]At the time the antibody results were obtained.
[b]Included both preformed and de novo DSA.
AZA, azathioprine; n.s., nonsignificant.

transplant recipients, patients with dnDSA versus no dnDSA were significantly less likely to be receiving tacrolimus (77% [50/65] vs 90% [162/174]; $P = 0.009$, respectively).[42] However, dnDSA is a frequent finding even in patients treated with standard-dose CNI. Everly et al[131] reported dnDSA in 20% of kidney transplant patients after 4 years despite standard-dose CNI with triple immunosuppression. In contrast, a prospective study of 90 liver transplant patients revealed no dnDSA at 4 months after transplantation in patients receiving a regimen of tacrolimus, MMF, and steroids,[51] a difference that may be due to the lower immunogenicity of liver grafts versus kidneys, but may also reflect the short 4-month follow-up period. The level of CNI exposure is likely to be important. Kaneku et al[6] retrospectively analyzed factors associated with dnDSA formation in 749 liver transplant patients at a single center, of whom 8.1% developed dnDSA within 1 year of transplantation. In multivariate analysis, patients with a low CNI trough concentration (tacrolimus <3 ng/mL or CsA <75 ng/mL) were at the highest risk of dnDSA formation (OR, 2.66; 95% CI, 1.2-5.84; $P = 0.015$). This is a potential cause for concern because these levels have been reported during the maintenance phase of some relatively aggressive CNI reduction studies in kidney transplantation.[132,133]

Discontinuation of CNI therapy early after kidney transplantation in the absence of suitable induction therapy may result in inadequate immunosuppression to prevent dnDSA formation.

Several studies in kidney and liver transplantation have reported that tacrolimus-based immunosuppression is associated with a lower risk of dnDSA formation than CsA-based regimens.[4,6,47,55,130] In 1 large single-center liver transplant experience, use of CsA versus tacrolimus was significantly associated with an increased risk of dnDSA (OR, 2.5; $P = 0.004$).[6]

Generally, the available data demonstrate a clear signal that compliance with CNI, and adequate CNI trough levels, play a more important role in dnDSA formation than the choice of CNI; however, in compliant patients tacrolimus may provide more protection from dnDSA formation than CsA.

Purine Synthesis Inhibitors and Antimetabolites

Maintenance immunosuppressive therapy with MPA profoundly depresses the primary and secondary humoral response in kidney transplant patients,[134,135] but data on the effect of purine synthesis inhibitors modulation of dnDSA formation are still inconclusive. Studies in kidney transplantation have shown inconsistent results regarding an effect of MMF versus azathioprine in terms of dnDSA onset[130,136] (Table 3). No conclusive results could be reached in a large prospective study by Hourmant and colleagues,[4] although azathioprine was more frequently used than MMF among patients with dnDSA whereas the converse was true in DSA-negative patients. Retrospective analyses in liver transplant populations have also shown mixed findings (Table 3). Until more robust data are available, one cannot definitively conclude an independent beneficial effect of MPA on dnDSA formation. Although the bulk of data may suggest a modest benefit, this may simply result from increased intensity of immunosuppressive regimens that include MPA therapy, rather than a specific mechanism to reduce the risk of dnDSA.

mTOR Inhibitors

Well-designed prospective studies to assess the relationship between mTOR inhibitors and risk of dnDSA are lacking (Table 4). A recent post hoc analysis was performed on 127 kidney transplant patients at a single center randomized to convert from CsA to a CNI-free everolimus-based regimen at 3 to 4.5 months after transplantation or to remain on CsA (ZEUS).[54] All patients received basiliximab induction, MPA and were started on oral steroids; by the end of the observation period, 59% of everolimus-treated patients and 62% of CsA-treated patients were steroid-free. During a median follow-up of 1273 days, dnDSA was detected in 23.0% of patients (14/61) who stopped CsA and switched to everolimus, compared to 10.8% of patients (7/65) who continued CsA (HR = 2.43; $P = 0.048$). The time to first detection of dnDSA was shorter in the everolimus cohort (median, 551 days vs 1173 days in the CsA group), and AMR occurred in 8 everolimus-treated patients compared to 2 CsA-treated patients ($P = 0.036$).[54] Five of the 8 AMR patients in the everolimus group received reduced-dose MPA and 2 were steroid-free; the 2 patients in the CsA group with AMR had no MPA or steroids. Although underpowered, everolimus monotherapy was associated with dnDSA and AMR (HR = 5.35; $P = 0.036$) but since immunosuppression appears to have been inadequate in many cases, interpretation is difficult.

Conflicting results have been reported (so far in abstract form only) by Sommerer et al[139] in patients who received basiliximab induction with CsA to month 3, and were randomized to continue standard CsA, switch to low-dose CsA with everolimus or convert to a CNI-free everolimus regimen, all with MPA and steroids (HERAKLES). At four years after kidney transplantation, the incidence of dnDSA was similar in the standard CsA group (16.7%), the CNI-free group receiving everolimus (17.9%), and the CsA-everolimus cohort (29.6%; P = n.s.).

Kamar et al[49] found no effect on dnDSA formation after converting patients from CNI to everolimus when the switch took place at a later time after transplantation (median, 22 months). In their single center case–control retrospective study, the incidence of dnDSA was compared over a mean of 35 months in a cohort of 61 patients.[49] Controls were matched for age, sex, induction therapy, and date of transplantation. All patients were DSA-free at the initial comparison, and at last follow-up, the proportion of patients with dnDSA was not significantly different between the everolimus group (9.8% [6/61]) and the CNI-treated controls (5% [3/61]; P = n.s.), with a similar median time to dnDSA detection (9.5 months and 13 months, respectively) and everolimus trough concentrations.[49] Another retrospective study[18] found a significantly higher rate of dnDSA in multivariable analysis of 56 kidney transplant patients converted from tacrolimus to mTOR monotherapy (sirolimus in 84%) at a mean of 1.3 years after transplantation compared to 214 who continued tacrolimus therapy. However, patients converted to sirolimus more than a year after transplantation had a similar rate of dnDSA emergence to the tacrolimus-treated cohort, once again suggesting that early mTOR inhibitor monotherapy conversion (<1 year) provides inadequate immunosuppression.[18] Prevalence data from a series of 267 maintenance kidney transplant patients observed no significant difference in mTOR inhibitor therapy between recipients with or without class I or class II dnDSA.[25] Perbos and colleagues[138] reported no increase in dnDSA onset in a series of kidney, liver, heart, and lung transplant recipients receiving everolimus with low-dose CNI about 6 years after transplantation, consistent with

TABLE 3.

Antimetabolite or purine synthesis inhibitor maintenance therapy: A comparison of the risk of dnDSA

Reference	Study design	Organ type	N	Follow-up	Maintenance regimen	Univariate or multivariate* analysis		Use of therapy according to presence/absence of dnDSA
						dnDSA	*P*	
Del Bello et al, 2014[47]	Retrospective Cohort study Single center	Liver	232	Median 36.5 mo	Various	n/a	n/a	MMF was used[a] in 90% of patients with dnDSA vs 71% of patients with no dnDSA ($P = 0.04$)
Kaneku et al, 2013[6]	Retrospective Cohort study Single center	Liver	749	1 y	MMF or none/AZA[b] ± CsA/TAC ± Sirolimus ± Steroids	OR = 1.00 MMF vs no MMF*	0.99	
Lachmann et al, 2006[130]	Prospective Cohort study Single center	Kidney	1043	>6 mo to 4 y	MMF or AZA or no antimetabolite CsA or TAC ± Steroids	39% AZA[c] 26% MMF[c] 19% none[c]	0.37	
Piazza et al, 2006[136]	Retrospective Cohort study Single center	Kidney	449	Not stated	MMF or AZA CsA Steroids	8% MMF 23% AZA	<0.0001	
Hourmant et al, 2005[4]	Prospective Cohort study Single center	Kidney	1229	5 y	Various	n/a	n/a	MMF was used[a] in 43%, 31% and 53% of patients with no antibodies, dnDSA or non-DSA ($P < 0.02$) AZA was used[a] in 31%, 43% and 23% of patients with no antibodies, dnDSA or non-DSA ($P < 0.02$)

Asterisk indicates analyses were performed with multivariate analysis.
[a]At the time the antibody results were obtained.
[b]363 patients received no induction, 9 patients received AZA.
[c]Included both preformed and de novo DSA.

TABLE 4.

mTOR inhibitor maintenance therapy: A comparison of the risk of dnDSA

Reference	Study design	Organ type	N	Time from transplant to Switch	Follow-up	Maintenance regimen	Univariate or multivariate* analysis dnDSA	P
Kaneku et al, 2013[6]	Retrospective Cohort study Single center	Liver	749	—	1 y	SRL (n = 119) or no SRL (n = 630) at 1 y ± MMF/AZA ± CsA/TAC ± Steroids	OR = 0.66* SRL vs no SRL	0.36*
Early switch (<1 y) to mTOR inhibitor								
Liefeldt et al, 2012[54]	Prospective Randomized[a] Single center	Kidney	127	3-4 mo	Median, 3.5 y	CsA or CsA converted to EVR (months 3–4.5) MPA Steroids	HR = 2.67* EVR vs CsA	0.04*
Late switch (>1 y) to mTOR inhibitor								
Croze et al, 2014[18]	Retrospective Cohort study Single center	Kidney	270	Mean, 1.3 y	Mean, 3.8 y	TAC[b] switched to SRL[c] ± Steroids to month 3	HR = 2.4* SRL vs TAC	0.04*
Ruiz San Millán et al, 2014[137]	Retrospective Cohort study Single center	Kidney	35	Median, 69 mo (range, 3-375)	2 y	CNI switched to mTOR inhibitor ± MMF/AZA ± Steroids	8.6% mTOR inhibitor 0% controls (n = 10)	n.s.
Perbos et al, 2014[138]	Retrospective Case control study Single center	Various	131	Mean, 73 mo	1 y	CNI switched to EVR ± CNI[d] or CNI ± MMF (controls)	6.7% EVR 11.1% controls	0.39

Asterisk indicates analyses were performed with multivariate analysis.
[a] Post hoc analysis of patients randomized at a single center within a multicenter study.
[b] 17 patients received CsA instead of TAC.
[c] 9 patients received EVR instead of SRL.
[d] 59% of EVR-treated patients also received low-dose CNI.
EVR, everolimus; mTORi, mammalian target of rapamycin inhibitor; SRL, sirolimus.

limited data in kidney transplant patients treated with mTOR inhibitors and low-dose extended-release tacrolimus.[140] In liver transplantation, Del Bello and colleagues[47] found no significant association between mTOR inhibitor therapy and dnDSA formation.

Taken together, existing data do not provide conclusive evidence that immunosuppressive regimens based on an mTOR inhibitor and reduced-exposure CNI therapy are invariably associated with increased risk of dnDSA formation. However, an early switch from CNI therapy to mTOR inhibitor monotherapy may increase the risk of dnDSA production, whereas late (>1 year) posttransplant conversion to mTOR inhibitor monotherapy may not increase the risk of dnDSA formation.

Corticosteroids

In an attempt to prevent or minimize steroid-associated side effects, some immunosuppressive strategies avoid the use of steroids or enable their early discontinuation (Table 5). In a prospective, single-center analysis by Lachmann and colleagues,[130] no significant difference in the development of dnDSA could be seen in kidney transplant patients with or without steroid therapy at the time of antibody testing. In a retrospective analysis of 749 liver transplant patients by Kaneku et al,[6] the risk of dnDSA was reduced in the presence of steroid therapy in univariate but not multivariate analysis. Similarly, in a retrospective analysis of 232 liver transplant patients tested annually for dnDSA, multivariate analysis did not show steroid therapy to be independently associated with dnDSA.[46] Despite consistent data, these findings are potentially confounded; steroids may have been preferentially discontinued in patients with well-functioning, rejection-free grafts.

Planned early steroid discontinuation may not influence the risk of dnDSA, depending on the immunosuppressive regimen used. Delgado and colleagues treated 37 kidney transplant patients with rATG induction, tacrolimus and MMF who were randomized to steroid withdrawal at week 1 posttransplant versus standard steroid therapy in a double-blind trial with annual follow-up of up to 5 years with no difference in dnDSA formation.[65] Consistent with these results, Li et al[66] reported no dnDSA with excellent graft function in 13 pediatric transplant patients at high immunological risk who received entirely steroid-free immunosuppression with a similar regimen (rATG induction with tacrolimus/MMF maintenance therapy).

Intriguing data regarding the influence of steroids comes from a series of 72 living-donor kidney transplant patients given a tolerance protocol comprising clonal depletion by total lymphoid irradiation or bortezomib followed by low-dose maintenance immunosuppression (steroids with or without one other agent).[141] Donor-specific antibodies were assessed every 1 to 2 months. At 3 months, patients tapered to less than 10 mg/day had a high rate of dnDSA (53%), compared to patients maintained on 10 to less than 20 mg/day (22%), and the lowest risk (0%) was seen in patients continued on 20 mg/day or greater or steroids plus another immunosuppressant agent. In multivariate analysis, steroid dose was inversely associated with dnDSA production, with an adjusted risk ratio of 0.92 (95%, 0.85-0.99; $P = 0.03$) for every 2.5 mg/day increase.

Thus, existing data present a complex picture regarding an association between steroid therapy and risk of dnDSA production. Current findings do not suggest an adverse effect of early steroid withdrawal or steroid-free immunosuppression in selected patients, but firm conclusions cannot be drawn.

FUTURE DIRECTIONS

Understanding how to appropriately regulate the B-cell compartment is critical to prevent dnDSA and achieve decades-long survival for solid organ transplants. Careful evaluation of the known impact of immunosuppressive agents on dnDSA has highlighted the major shortcomings of existing reports. As a consequence, we are currently unable to "personalize" immunosuppressive treatment to prevent dnDSA.

New studies should consider the addition of novel anti–B-cell agents that have recently emerged in other relevant fields, such as hematopoietic stem cell transplantation,[142] autoimmunity,[143] and multiple myeloma.[144] However, careful target selection may be critical to success because simple B-cell annihilation is likely not the answer given the difficulty of reaching niche resident plasma cells and the critical importance of regulatory B-cells.[145]

Recently, several new interesting B-cell–directed strategies have attracted attention, which can be divided into direct and indirect B-cell agents.[143] Novel direct B-cell agents include variants of anti-CD20 monoclonal antibodies (mAbs), such as *ocrelizumab*, which more thoroughly deplete B-cells,[146] and anti-CD19 mAbs that also deplete memory B-cells and short-lived plasma cells while $CD19^-$ plasma cells remain unaffected.[147] Furthermore, the anti-CD22 mAb *epratuzumab* is able to modulate B-cell function by altering adhesion molecule expression, interfering with migration,[148] and inhibiting BCR-dependent B-cell activation.[149]

Indirect B-cell targeting can be accomplished through modulation of the BAFF/APRIL pathway, primarily produced by macrophages, neutrophils, and dendritic cells and also B-cells and activated T-cells, comprising 3 cellular receptors (BAFF-R, BCMA, and TACI) differentially expressed by different subpopulations of B-cells.[150] In particular, BAFF-R is critical for immature B-cell survival/maturation, BCMA is required for plasma cell survival, and TACI is necessary for T-cell–independent B-cell responses, regulation of B-cells and Ig class-switching. Therefore, the BAFF/APRIL pathway appears to lie at a critical immune intersection that may regulate the B-cell compartment. Accordingly, targeting such a complex system with novel and specific interventions has gained considerable interest. Kwun and colleagues[151] recently documented simultaneous neutralization of BAFF and APRIL using a fusion protein composed of the TACI receptor and Ig Fc (TACI-Ig, atacicept), which prevented early DSA formation and AMR in a depletion-induced preclinical AMR model.

Plasma cell targeting represents another area of active research. Interference with BAFF/APRIL using the anti-BAFF mAb *tabalumab* is being explored.[152] In addition, second-generation proteasome inhibitors are being evaluated[153] and other plasma cell depleting agents that target cell surface molecules (CD38 and CD138) are currently under investigation.[144] In addition, some bortezomib-recalcitrant multiple myeloma patients have responded to novel proteasome inhibitors targeting different sites.[154] Additionally, research in the complex ubiquitin proteasome system is generating novel molecules that target protein degradation upstream of the proteasome and drug combinations with enhanced therapeutic capacity. Finally, orally available proteasome inhibitors may soon become available.[155]

An effective B-cell response is also considerably influenced by the critical contribution of helper T-cells. As a consequence, novel costimulatory blockers, such as CTLA4-Ig

TABLE 5.

Steroid maintenance therapy: A comparison of the risk of dnDSA

Reference	Study design	Organ type	N	Follow-up	Maintenance regimen	Univariate or multivariate* analysis		Use of Therapy according to presence/absence of dnDSA
						dnDSA	*P*	
Del Bello et al, 2014[47]	Retrospective Cohort study Single center	Liver	232	Median 36.5 mo	Various	n/a	n/a	Steroids were used[a] in 19% of patients with dnDSA 19% and 44% of patients with no dnDSA ($P = 0.02$)
Kaneku et al, 2013[6]	Retrospective Cohort study Single center	Liver	749	1 y	Steroids or no steroids ± CsA/TAC ± MMF/AZA ± Sirolimus	OR = 0.67* steroids vs no steroids	0.23*	
Hoshino et al, 2012[141]	Prospective Cohort study Single center	Kidney	72	1 y	Clonal depletion Steroids ± CNI, MMF or sirolimus	ARR = 0.92 per 2.5 mg/day increase in steroid dose	0.03	
Delgado et al, 2009[65]	Prospective Double-blind Randomized Single center	Kidney	37	≤5 y	TAC MMF Steroid withdrawal at day 7 (n = 21) or standard steroids (n = 16)	0% steroid withdrawal 6.3% standard steroids	0.43	
Lachmann et al, 2006[130]	Prospective Cohort study Single center	Kidney	1043	>6 mo to 4 y	Steroids or no steroids CsA or TAC ± MMF/AZA	27% steroids[b] 33% no steroids[b]	0.44	
Hourmant et al, 2005[4]	Prospective Cohort study Single center	Kidney	1229	5 y	Various	n/a	n/a	Steroids were used[a] in 22%, 40% and 43% of patients with no antibodies, dnDSA or non-DSA (n.s.)

[a]At the time the antibody results were obtained.
[b]Included both preformed and de novo DSA.
ARR, adjusted risk ratio.

(*belatacept*), that prevent T-cell help to B-cells may represent an important adjunct to prevent dnDSA. It should be noted, however, that an inhibitory role of belatacept on regulatory T-cells has been reported,[156] and in 1 study resulted in an increased risk of cell-mediated rejection.[157] Because regulatory T-cell generation is independent of the CD40-CD154 pathway, an anti-CD40 mAb may represent an alternative.[158]

Although cellular destruction has remained the dominant mechanism to treat dnDSA formation, prevention remains a superior approach. Interference with cellular trafficking to essential locations for costimulation has the potential to prevent dnDSA while allowing the regulatory cell populations to remain intact. Despite a lack of data and the withdrawal of efalizumab from the market, this type of approach remains attractive.[159]

Taken together, these data point to a large number of novel immunosuppressive agents that, in theory, hold the potential to prevent dnDSA. If combined with a better understanding of how to appropriately risk-stratify patients to receive these potent immunosuppressive agents, this critical combination of knowledge may be able to substantially decrease the risk of dnDSA, resulting in improved allograft survival in the not-too-distant future. However, to achieve this, we need well-designed prospective, multicenter, randomized clinical trials across organ transplant types that collect detailed donor and recipient genetic information, immunosuppression serum levels, and compliance data in addition to frequent HLA and non-HLA DSA testing[160,161] with different risk-based immunosuppressive strategies.

CONCLUSIONS

De novo DSA risk reduction is essential to prevent chronic AMR and improve long-term graft survival in all solid organ transplant patients. Utilization of solid-phase single-antigen assays as part of risk-based monitoring for circulating DSA has increased. However, personalizing immunosuppression precisely based on an individual's risk for dnDSA production remains in development.

Patients with a history of nonadherence, greater HLA mismatching (particularly for class II DQ or DR) or an increased propensity to sensitization due to previous transplant, transfusion, pregnancy, or previous acute rejection, are more likely to develop dnDSA. These individuals may need more intensive immunosuppression than those without risk factors. Especially in the first year after transplantation, rATG induction appears to attenuate dnDSA production in moderately sensitized patients. Administration of newer agents, such as rituximab or bortezomib, as induction therapy in at-risk patients may be of interest, but the benefits are unproven in adequately powered studies. Early CNI withdrawal, especially in the absence of depleting induction, is not advisable in patients with risk factors for dnDSA. Although late CNI withdrawal in lower-risk patients can be undertaken, close DSA surveillance is recommended. Mammalian target of rapamycin inhibitor monotherapy early after transplant is not recommended, but the combination of an mTOR inhibitor with reduced-exposure CNI has not been associated with increased risk of dnDSA. The available data do not indicate a consistent effect of MPA on dnDSA production. Early withdrawal of steroid therapy appears feasible with no increased risk of dnDSA when combined with induction and nonsteroid-based maintenance immunosuppression. Overall, the priority in at-risk individuals is to maintain adequate immunosuppression, establish an appropriate DSA monitoring protocol, and enforce adherence. The future demands DSA monitoring to become a routine component of randomized immunosuppression trials in all organs to improve our understanding of the relative risk of dnDSA production between regimens.

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