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REVIEW ARTICLE

The possible critical role of T-cell help in DSA-mediated graft loss

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SUMMARY

In this review, we discuss a possible central role of T-cell help in severe forms of graft damage mediated by donor-specific HLA antibodies (DSA). Some kidney transplant recipients with pretransplant DSA show a high graft failure rate, whereas in other patients DSA do not harm the transplanted kidney and in most cases, disappear shortly after transplantation. Analyzing 80 desensitized highly immunized kidney transplant recipients and another multicenter cohort of 385 patients with pretransplant HLA antibodies, we reported recently that an ongoing T-cell help from an activated immune system, as measured by an increased level of soluble CD30 in serum, might be necessary for the DSA to exert a deleterious effect. Patients positive for both pretransplant DSA and sCD30 appear to require special measures, such as the elimination of DSA from the circulation, potent immunosuppression, good HLA-matching, and intense post-transplant monitoring, whereas exclusion of DSA-positive patients from transplantation in the absence of high sCD30 may not be justified in all cases, even if the pretransplant DSA are strong and complement-activating.

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Introduction

Antibodies are Y-shaped immunoglobulin molecules that consist of two long heavy and two short light chains and form a Fc, a hinge, and two antigen-binding Fab regions. While the Fc and hinge regions contain only the constant parts of the heavy chain, Fab regions consist of variable and constant parts of the light as well as heavy chains and each of these chains form three variable complementary determining regions (CDR)1, CDR2, and CDR3 that are responsible for specific binding to an antigenic epitope that is present, for example, on a target cell in the transplanted foreign tissue. The CDR3 regions show the highest variability and herewith the best complementarity for the antigen. Subsequent to antigen binding, the Fc part of the immunoglobulin molecule can activate complement and/or attract complement- as well as Fc receptor (FcR)-carrying macrophages and natural killer cells that are capable of killing target cells by antibody-dependent cellular cytotoxicity (ADCC). Strong complement activation may also result in direct lysis of the cells after formation of the membrane attack complex C5b-9, which is capable of inducing pores in the membrane of the target cell.

Antibody molecules are produced by plasma cells which represent an advanced stage in the B-cell development. If the B cell receives, also enhanced by the CD40-CD40 ligand interaction, cytokine support from a

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corresponding T helper cell after antigen contact, it can become a plasma cell which is capable of producing up to 2.000 antibody molecules per second. And if under an ongoing immune response the T-cell help continues, the affinity of produced antibodies increases with each immunoglobulin recombination cycle as a result of somatic hypermutations and formation of CDR regions which fit better to the antigenic epitope. Furthermore, depending on the type of T-cell help and released cytokines, immunoglobulin subclasses are produced with Fc regions that activate complement and/or strongly bind FcR. If the T-cell support is fully interrupted, for example, by immunosuppression, *de novo* antibody production stops, whereas previously activated plasma cells may continue antibody secretion for a certain period in a T-cell-independent manner. To understand the central role of the T cell in antibody production and antibody-mediated kidney transplant rejection (AMR), a closer look is necessary into the generation of an antibody response.

Recognition of foreign tissue by the recipient's immune system and generation of an antibody response

For the generation of an effective antibody response to a kidney allograft, T-lymphocyte help to B cells, enhanced by the CD40-CD40 ligand pathway, is required [1]. However, before this happens, T lymphocytes (CD4+ and CD8+ cells) are primed either by recognition of mismatched HLA antigen epitopes on donor antigen-presenting cells (APC) (direct allorecognition) or by peptides derived from processed foreign HLA molecules expressed on antigen-presenting dendritic cells of the recipient (indirect allorecognition) [2,3]. It is generally acknowledged that, if suppression of the T cell-dependent immune response by immunosuppressive agents is not sufficient, both (direct and indirect) pathways are active during the whole lifespan of the graft, contributing to chronic organ transplant injury [4,5]. More recently, the existence of the semidirect pathway was suggested, according to which alloantigen is internalized and without being processed, is presented on the cell surface [6]. B cells as APC are themselves involved in antigen presentation. If they recognize and bind an antigenic epitope with their surface immunoglobulin receptor, this antigen molecule can be internalized, processed, and presented with the help of MHC class II molecules in form of peptides to T helper (Th) cells, typically to follicular Th (Tfh) cells.

Considerable evidence has accumulated during the last years on the central role of Tfh cells in the initiation of antibody production and development of AMR. If follicular B cells (antigen-activated naive as well as reactivated memory B cells) recognize a foreign antigen in the germinal center of secondary lymphoid organs, such as lymph nodes or spleen, they move to the T-cell border and present the antigen they recognized to Tfh cells (Fig. 1). Supported by Tfh help in the form of IL-21 and IL-4, B cells differentiate into either short-lived plasma cells producing low-affinity IgM antibodies or, after cycles of somatic hypermutations, and isotype switch (affinity maturation) into memory B cells /or long-lived plasma cells secreting high-affinity IgG antibodies [7]. B cells that are localized in the marginal zone of lymphoid organs, on the other hand, have the function to filter antigen from the blood to form a T-cell-independent first response. In kidney biopsies taken during rejection, Tfh cells colocalized with B cells and immunoglobulins were found in follicular-like lymphoid aggregates (tertiary lymphoid organs), and higher numbers of circulating Tfh cells were reported in patients with pre-existing donor-specific HLA antibodies (DSA) at 3 months after transplantation [8].

Pretransplant recognition of patients with high alloreactivity

The majority of kidney transplantations are performed against HLA mismatches and an increasing number, after desensitization, against a DSA barrier (HLA-incompatible transplantation). Therefore, the precise characterization of DSA and the pretransplant measurement of the organ recipient's immune state against a specific donor and recognition of patients with an increased risk of AMR are becoming increasingly important. Currently, the routinely used way to determine a patient's alloreactivity is the measurement of DSA. However, not all patients with DSA lose their grafts. Before 2000, when DSA determinations by the highly sensitive single antigen bead (SAB) technology were not routine, a high number of kidney transplantations were performed with a negative lymphocytotoxic crossmatch but in the presence of DSA, and many of these transplants are still functioning.

We recently obtained and published evidence from two independent studies that T-cell help from a preactivated immune system is necessary for the deleterious action of SAB-detected pretransplant DSA that went undetected in the lymphocytotoxic crossmatch. In the first study, the immunological and histological

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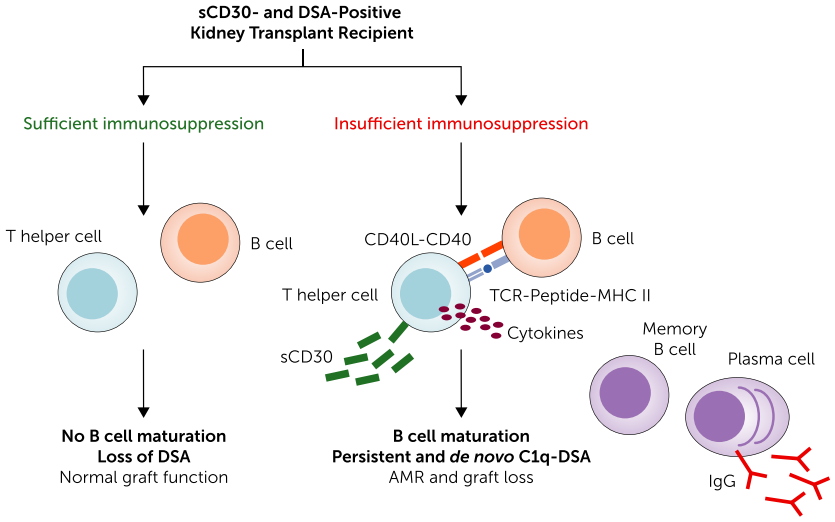

Figure 1 Development of graft loss under insufficient immunosuppression due to antibody-mediated rejection (AMR) in patients with donor-specific HLA antibodies (DSA) and T-cell help from a preactivated immune system before transplantation.

characteristics of patients who lost their graft due to antibody-mediated rejection were analyzed [9]. These patients were categorized as high risk according to the Heidelberg approach for transplantation of high-risk sensitized patients [10] and were transplanted under several measures, such as desensitization, potent immunosuppression including rituximab and anti-T cell antibody induction, protocol biopsies, and post-transplant DSA monitoring. To confirm the results obtained in this study, in which pre- as well as serial post-transplant sera were available in 74 of 80 patients, pretransplant sera of additional 385 presensitized patients from the multicenter CTS serum study were studied separately [11]. In this second cohort, patients were transplanted without knowledge of the DSA presence so that the impact of pretransplant DSA could be analyzed without the interference of desensitization. Presence of donor as well as recipient DNA allowed reliable detection of DSA against 10 different HLA loci.

In both studies, pretransplant DSA were associated with graft loss only in patients with high pretransplant levels of the immune activation marker sCD30 [9,11]. In patients with low sCD30, the DSA did not harm the transplant. Even in high-risk kidney transplant recipients that were analyzed in the first study, the majority of DSA, even if C1q-binding, disappeared after kidney transplantation [9], indicating that desensitization and potent immunosuppression were effective in controlling the T cell-dependent B-cell response in many cases; preexisting DSA were not detectable in the patient's circulation anymore post-transplant. However, under insufficient immunosuppression, DSA may either persist or reappear again in patients with a preactivated immune system and the patient may develop *de novo* DSA. Although weak *de novo* DSA may damage the transplant in the long run, strong DSA may activate complement and cause immediate injury [9,12]. These observations strongly imply that the B-cell response requires continuous T-cell support to create an effective antibody production, especially during an acute humoral rejection process with the production of high-affinity IgG antibodies that are capable of damaging the transplant severely.

Elevated sCD30 as a marker of increased T-cell help

Elevated sCD30 may indicate an increased immunological capacity to react against foreign HLA antigens. Under physical conditions, the 120 kD glycoprotein CD30, as a member of the tumor necrosis factor (TNF) family, is expressed on activated T, B, and NK cells. Its ligand CD153 also belongs to the TNF receptor family and is found to be expressed on activated T cells, resting and malignant B cells, granulocytes, monocytes, macrophages, histiocytes, a fraction of bone marrow myeloid precursors, erythroblasts, and subsets of megakaryocytes. Polyclonal and allogeneic stimulation of T cells results in upregulation of CD30 on the memory subset of CD4+ and CD8+ T-cells [13]. During further course, CD30 is released into the blood stream mainly from memory CD4+ and to a lesser extent from memory CD8+ T cells through splicing by zinc metalloproteinases ADAM10 and ADAM17/TACE (TNF-α converting enzyme). Activated effector and memory T cells were shown to contribute to circulating sCD30 during the rejection process of islet allografts [14]. The *in vitro* sCD30-release could be inhibited by antibodies

that block T-helper cell cytokines [13], suggesting that high sCD30 serum levels reflect increased action of T helper cells. The released 85 kD sCD30 molecule is quite stable in serum and its concentration can easily be measured by ELISA [15].

During T-cell activation, CD4+CD3—CD11c— accessory T cells provide OX40- and CD30-dependent survival signals to follicular T cells. Hereby, OX40 and CD30 signals share common pathways and can substitute each other. CD30/CD30 ligand signaling plays an important role in the generation of long-lived memory CD8+ T cells and the same pathway was shown to be a potent regulator of CD4+ T cell-mediated graft versus host disease (GVHD) [16]. Under insufficient immunosuppression, the T-cell help exerted by Tfh cells in lymph nodes and the involvement of CD30+ T cells and sCD30 production would need further experimental clarification. Importantly, mice deficient in OX40 as well as CD30 were reported to lack memory antibody responses [17].

Increased serum concentrations of sCD30 are linked to presensitization; patients with HLA antibodies and previous transplantations show higher sCD30 levels [15]. Due to contact with bioincompatible dialysis membranes, foreign HLA or HLA cross-reactive infectious agents [18], end-stage renal disease patients exhibit alterations in their immune response, including increased numbers of interferon (INF)-γ-producing HLA-cross-reactive memory T-cells, reduced numbers of regulatory T-cells, augmented *in vitro* expansion of T-cells expressing CD30, and an increased serum sCD30 content [13,19]. We reported that cytomegalovirus infection can also lead to an increase of serum sCD30 levels in transplant patients [20] and Chan *et al.* identified CD30-positive T-cells as the major INF-γ and IL-5 cytokine-producing human T-lymphocyte subset generated in response to stimulation with alloantigens [21]. High production of IFN-γ by alloreactive antidonor effector memory T-cells in patients awaiting kidney transplantation, as measured by ELISPOT, has been associated with early rejection of kidney transplants [22] and neutralization of IFN-γ results in abrogation of sCD30 release from memory T-cells *in vitro* [13]. Moreover, increased pre- as well as post-transplant levels of sCD30 were associated in many studies with an increased risk of graft loss [15,20–24]. Our studies indicated that a serum level between 80 and 100 ng/ml can be used as a clinically meaningful cutoff for tests before transplantation and 40 ng/ml as a cutoff for tests after transplantation. Interestingly, patients with acute GVHD also possess high serum levels of sCD30 and an increased percentage of CD30-expressing CD8+ central memory T cells [25].

Possible functions of sCD30

Although a biological function of sCD30 has not been clearly established *in vivo*, sCD30 has greater affinity for CD30-ligand *in vitro* as compared to CD30 and is potentially capable of blocking CD30/CD30-ligand interactions [26], reducing the availability of CD30-ligand on lymphocytes or decreasing the ability of regulatory T-cells to inhibit graft-reactive T-cells [27]. CD4+CD25+ regulatory T cells were shown to suppress allograft rejection mediated by memory CD8+ T cells via a CD30-dependent mechanism [28]. High concentrations of sCD30 may block this inhibitory mechanism and result in higher alloreactivity. Alternatively, high sCD30 levels in the circulation may simply reflect an activated immune system and increased presence of CD30+ memory T cells and Tfh cells that produce cytokines as T-cell help for B cells [14].

Highly sensitized kidney transplant recipients with DSA-mediated graft loss showed increased pretransplant serum levels of sCD30

As mentioned above, we analyzed the combinatory effect of the B-cell marker DSA and the T-cell activation marker sCD30 in two independent patient collectives. The first collective consisted of 80 highly sensitized kidney transplant recipients who were transplanted under potent immunosuppression and desensitization [9]. In this unique cohort of patients, the immunological processes occur in an accelerated manner and despite all measures, six patients lost their graft due to AMR within the first 3 years after transplantation. The retrospective analysis of immunological parameters, such as pretransplant crossmatch results, pre- and post-transplant DSA, C1q-binding capability of DSA and pre- and post-transplant levels of the T-cell activation marker sCD30 in AMR cases revealed interesting results. Detection of pretransplant DSA predicted post-transplant graft loss from AMR with high sensitivity but low specificity. Addition of the C1q-binding capacity of DSA did not further improve pretransplant prediction of graft loss as pretransplant C1q-DSA were lost post-transplant in as many as 11 of the 13 high-risk patients. In contrast, persistence or *de novo* occurrence of C1q-DSA after kidney transplantation was associated with AMR and subsequent graft loss in six of seven patients [9].

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Importantly, high-risk patients with pretransplant positivity of the activation marker sCD30 had a 7 times higher risk of post-transplant graft loss ($P = 0.033$) than patients who were sCD30 negative before transplantation. Prediction of post-transplant graft loss was further improved when pretransplant DSA and pretransplant sCD30 were considered in combination: post-transplant graft loss from AMR (AMR-GL) was more than 11 times higher in patients who were DSA and sCD30 positive pretransplant than in all other patient groups ($P = 0.013$). A patient who was DSA positive but sCD30 negative before transplantation and suffered from AMR-mediated graft loss after surgery had a gap in immunosuppressive therapy at the time of AMR and became sCD30 positive during the post-transplant phase [9], indicating a possible *de novo* activation of alloreactive T-cells. Figure 2 demonstrates the kinetics of sCD30 and DSA in these six patients along with other clinical events.

These findings suggested that pretransplant identification of patients who are positive for the T-cell activation marker sCD30 together with the B-cell activation marker DSA may help preventing antibody-mediated graft loss in highly sensitized patients who are particularly prone to post-transplant antibody-mediated allograft injury. We hypothesized that persistence or *de novo* occurrence of C1q-DSA with subsequent AMR-GL depends on the T-cell help from an activated immune system as indicated by increased serum levels of sCD30.

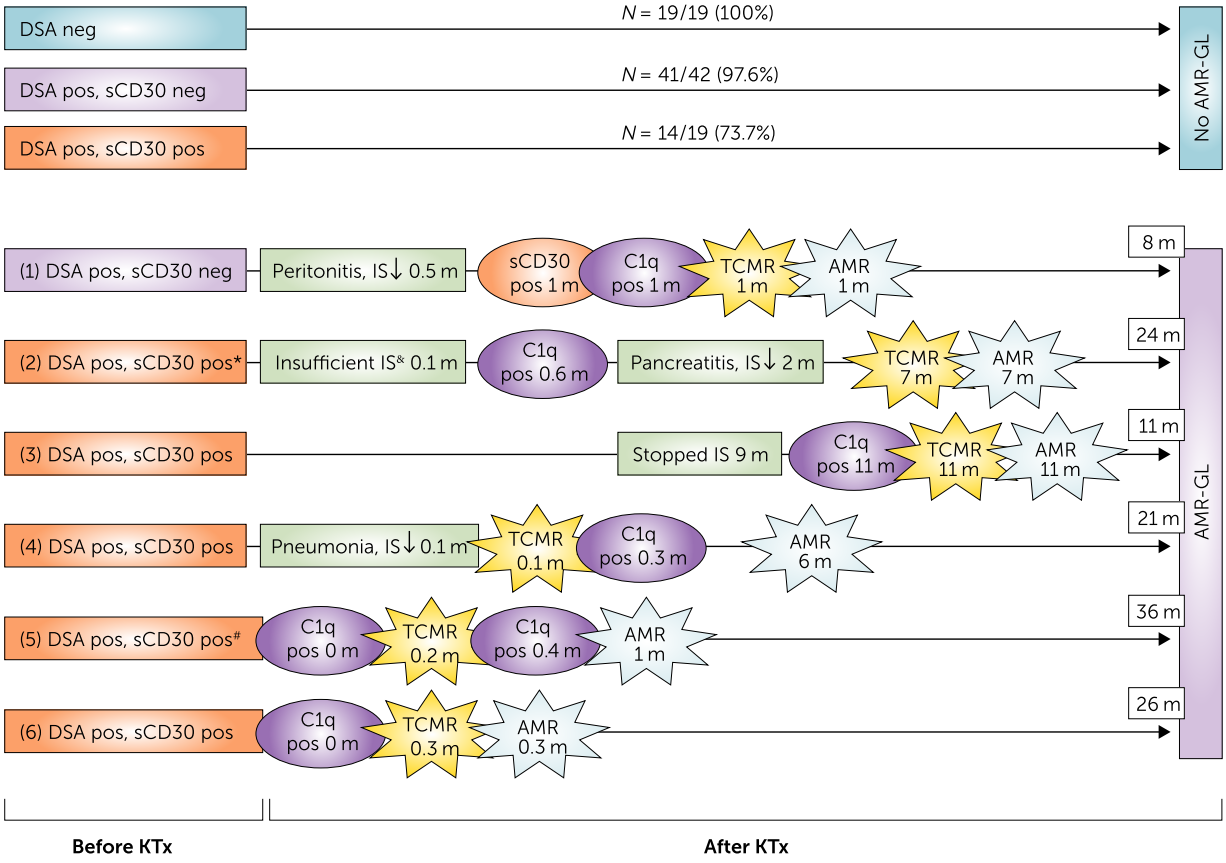

Figure 2 Influence of pretransplant donor-specific antibodies (DSA) and soluble CD30 (sCD30) on antibody-mediated rejection (AMR)-related graft loss (AMR-GL) (cases published in Ref. 9). In this desensitized immunologically high-risk collective from the Heidelberg transplant center, AMR-GL occurred mainly in patients with pretransplant positivity for DSA and sCD30 (patients 2-6). Only one patient with sCD30 negativity (patient 1) suffered from post-transplant AMR-GL. This patient was on minimal immunosuppression (IS) directly after kidney transplantation (KTx) due to peritonitis and sepsis and became sCD30 positive one month after surgery with concomitant C1q-DSA (C1q) development and T-cell-mediated rejection (TCMR) and AMR. Two patients with early AMR had either persistence of pretransplant C1q-DSA (patient 6) or persistence and *de novo* occurrence of C1q-DSA (patient 5) with subsequent AMR-GL. *This sCD30-positive patient was judged sCD30-negative in an earlier publication due to the application of a higher cutoff of 100 U/ml (Ref. 9). m, months, #patient with persistence and *de novo* occurrence of C1q-DSA, &patient with insufficient immunosuppression due to postoperative intestinal resorption failure and recurrent vomiting.

Pretransplant DSA had a significantly deleterious impact on graft survival only in the presence of high pretransplant levels of the T-cell activation marker sCD30

Because the number of patients with AMR-GL was with six quite low in our local high-risk collective, we tested the combined effect of DSA and sCD30 also in a multicenter effort that was initiated within the Collaborative Transplant Study. We studied 385 presensitized adult recipients of deceased donor kidney transplants with HLA antibodies who were transplanted during 1996–2011 without special measures and on whom we received, besides a pretransplant serum, also recipient and donor DNA which allowed reliable determination of DSA against 10 different HLA loci. The results of this study were published in the July 2016 issue of EBioMedicine [11].

Similar to the results obtained in our first study, a deleterious influence of pretransplant DSA on graft survival was again evident only in patients who were positive for the immune activation marker sCD30. In the absence of sCD30 positivity, the outcome was virtually identical in patients with or without DSA. In contrast, patients who were sCD30 as well as DSA positive demonstrated an extremely poor 3-year graft survival rate of 62% and showed in the multivariate analysis a 2.9-fold higher risk of graft loss within the first 3 years than the DSA and sCD30 negative patients ($P < 0.001$) [11]. As shown in Fig. 3, even the presence of strong pretransplant DSA with ≥5 000 MFI was not associated with inferior outcome in the absence of sCD30. DSA and sCD30 positive patients, in contrast, demonstrated a very low 5-year graft survival of 49%.

Clinical relevance of sCD30-DSA findings

One would have expected that the presence of DSA at the time of transplantation would be harmful to the graft, in any case, if not via complement activation, then through ADCC which, as mentioned above, can induce tissue damage by activating macrophages and NK cells. This was not the case and only less than 40% of the DSA-positive patients were sCD30 positive in our EBioMedicine study and showed inferior outcomes. Some transplant centers perform transplantations after the elimination of DSA by plasma exchange or immunoadsorption, combined with potent immunosuppression which often includes the B- and T-cell-eliminating agents rituximab and rabbit antithymocyte globulin (rATG), with variable success [10,29]. Our study suggests that treatment measures with strong side effects and high costs may not be in all cases necessary in pretransplant DSA-positive patients with a low serum sCD30. However, because reported so far only by us, this finding should be considered preliminary and needs to be substantiated by further investigations and, if confirmed, directly applied to the clinical routine.

Despite the presence of DSA, many patients possess low sCD30 levels which can be explained by the presence of a low-level or declining effector/memory T-cell response. The precise relationship between alloreactive T cells and sCD30 still needs to be demonstrated *in vivo*, for example, by parallel measurement of sCD30 in ELISA and donor-specific IFN-γ producing alloreactive T cells, for example, by ELISPOT [22]. For the use in clinical routine, the ELISPOT test is in contrast to sCD30 measurement donor-specific, but on the other hand, more laborious and the missing donor-specificity in the case of sCD30 is compensated by the detection of DSA. In the BIO-DrIM consortium effort, the IFN-γ-ELISPOT is currently used to assess the antidonor memory/effector T-cell alloresponse to perioperatively stratify transplant patients into low and high responders [30]. We are utilizing sCD30 routinely in the

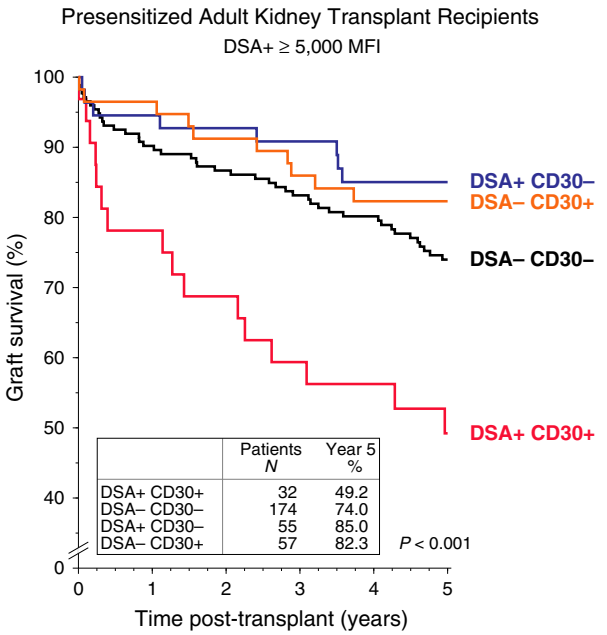

Figure 3 Impact of pretransplant DSA and sCD30 on graft survival (cases published in Ref. 11). Patients with high DSA of ≥ 5 000 MFI show good survival rates in the absence of high pretransplant sCD30 (<80 ng/ml), whereas graft outcome is extremely impaired in patients with high DSA and high pretransplant sCD30.

adjustment of immunosuppression in our immunologically high-risk patients.

Donor-specific antibodies of patients with high sCD30 levels appear to be able to exert, most probably, due to a higher antigen affinity and strong C1q-binding, more severe tissue damage. IgG subclasses, complement binding capacities, and affinity of DSA in sCD30-positive and sCD30-negative patients, however, need to be analyzed in more detail. Demonstration of the histopathological differences at the protein and transcript level in biopsy samples of AMR patients with and without sCD30 could also be useful. Hirt-Minkowski *et al.* reported a strong correlation between serum sCD30 levels and tubulointerstitial inflammation in biopsy samples of patients with clinical rejection, whereas such an association was missing in subclinical rejection [31]. In contrast, Grenzi *et al.* detected a higher rate of subclinical rejection in protocol biopsy samples of patients who were on low dose tacrolimus and had increased post-transplant sCD30 serum levels [32]. In our desensitized high-risk collective from Heidelberg, biopsies of patients with AMR-GL showed in all cases T-cell-mediated rejection phenotypes, either preceding biopsies with the AMR phenotypes (patients 4 and 5) or concomitant T-cell-mediated rejection at the diagnosis of AMR (patients 1, 2, 3, and 6). Five of six patients with AMR-GL had C4d-positive biopsies. Currently, applied potent immunosuppression appears to be able to control low-level T-cell-mediated antibody responses. In contrast, special measures, such as the elimination of DSA from the patient's circulation, potent immunosuppression, including antibody induction, good HLA-matching, and intense post-transplant monitoring [10,33,34], are required in pretransplant DSA-positive patients with a preactivated immune system.

In conclusion, there is strong evidence that the pretransplant presence of DSA in conjunction with a strongly activated immune system and T-cell help, as measured by sCD30, increases the risk of AMR-mediated graft damage by DSA that may possess in this constellation higher antigen affinity and stronger capacity of complement activation. Polyclonal rATG binds to the CD30 molecule [35], and in the mixed lymphocyte culture, the sCD30-release was inhibited by antibodies that block T-helper cell cytokines IL-2 as well as IFN-γ [13]. These findings underline the observed beneficial effect of induction therapy with rATG and IL-2-receptor antagonists in immunologically high-risk patients [10,36]. Anti-T cell antibodies could further be combined in DSA-sCD30-positive cases with complement-inhibiting and/or plasma cell-eliminating agents, to prevent the damage induced by complement-activating DSA and inhibit ongoing DSA production by already activated plasma cells, respectively. We are currently investigating whether elimination of CD30+ T cells by an anti-CD30 antibody can be helpful to inhibit an ongoing strong alloimmune response.

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Conflict of interest

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