Review

Soluble CD30 serum level - an adequate marker for allograft rejection of solid organs?

G. Schlaf, W.W. Altermann, A. Rothhoff and B. Seliger

Institute of Medical Immunology, Tissue Typing Laboratory, Martin-Luther University Halle-Wittenberg, Halle/Saale, Germany

Summary. The CD30 molecule, a 120 kDa cell surface glycoprotein, is a member of the tumor necrosis factor receptor (TNF-R) superfamily and was originally identified on the surface of Reed-Sternberg cells and anaplastic large cell lymphomas in Hodgkin's disease patients. In addition to lymphoproliferative disorders the expression of CD30 was found in both activated $CD8^+$ and $CD4^+$ Th2 cells which lead to the activation of B-cells and consequently to the inhibition of the Th1-type cellular immunity. The membrane-bound CD30 molecule can be proteolytically cleaved, thereby generating a soluble form (sCD30) of about 85 kDa. Low serum levels of soluble CD30 were found in healthy humans, whereas increased sCD30 serum concentrations were detected under pathophysiological situations such as systemic lupus erythematosus, rheumatoid arthritis, certain viral infections and adult T cell leukaemia/lymphoma. In addition, it has recently been suggested that pre- or post-transplant levels of sCD30 represent a biomarker for graft rejection associated with an impaired outcome for transplanted patients. We here review (i) the current knowledge of the clinical significance of sCD30 serum levels for solid organ transplantations and (ii) our own novel data regarding inter- and intra-individual variations as well as time-dependent alterations of sCD30 levels in patients. (iii) Based on this information the implementation of sCD30 as predictive pre-transplant or post-transplant parameter for solid organ transplantation is critically discussed.

Key words: Allograft transplantation, Enzyme-linked immunosorbent assay (ELISA), Graft rejection, Human leukocyte antigen (HLA), Soluble CD30 (sCD30)

Introduction

The CD30 molecule which was first described in 1982 as the antigen of the monoclonal antibody Ki-1 (Schwab et al., 1982; Stein et al., 1982) represents a 120 kDa transmembrane glycoprotein belonging to the tumor necrosis factor receptor (TNF-R) superfamily (Froese et al., 1987; Nawrocki et al., 1988; Smith et al., 1993, 1994). The soluble form (sCD30) is produced by metalloprotease cleavage of the extracellular domain, thereby releasing an approximately 85 kDa protein (Josimov-Alasevic et al., 1989). Membrane-bound CD30 expression was primarily discovered on Reed-Sternberg cells of Hodgkin lymphomas (Schwab et al., 1982), but later also on other malignant and normal cells (Stein et al., 1985). Furthermore, sCD30 only detectable at low levels in most normal individuals was elevated in pathophysiological conditions, such as $CD30^+$ hematopoietic malignancies, certain viral infections and inflammation processes. Recently, sCD30 serum levels were also determined prior or post solid organ transplantations in order to define their clinical relevance as a predictive marker for the allograft rejection of solid organs.

Physiological expression and function of CD30, sCD30 and CD30-ligand

The expression of CD30-ligand (CD30L) and CD30 is restricted to cells of the immune system and strictly regulated under physiological conditions. CD30 is not expressed in resting, but in diverse activated immune cells including $CD4^+$ and $CD8^+$ T lymphocytes (Ellis et al., 1993; Bengtsson et al., 1995; Agrawal et al., 1996; Sun et al., 2001; Stanciu et al., 2001), in particular on activated CD45RO Th2 cells, B cells (Shanebeck et al., 1995), natural killer (NK) cells (Cambiaggi et al., 1993), dendritic cells (DC) (Pellegrini et al., 2005), as well as some cells of non-lymphoid origin (Duerkop et al., 2000). Only <2% of human peripheral blood mononuclear cells (PBMNC) from healthy individuals bear the CD30 receptor (Del Prete et al., 1995).

Regarding its activity, CD30 regulates cell proliferation, differentiation and apoptosis, but its biological activity is dependent on the type and developmental stage of cells. Its function in mature PBMNC is not well understood, but there exists some evidence that CD30 can act as a signal transduction molecule (Muta et al., 2000).

Soluble CD30 is cleaved from the surface of CD30$^+$ cells by the cell surface metalloproteinase TNF-α converting enzyme (TACE) (Hansen et al., 2000). CD30$^+$ cells release sCD30 *in vitro* and *in vivo* resulting in low sCD30 levels in the sera of most healthy individuals. Although the mechanisms leading to the release of sCD30 have not been studied in detail it might be a consequence of appropriate activation signals in response to interaction with CD30L$^+$ cells. The CD30 ligand (CD30L) is a transmembrane protein and member of the TNF ligand superfamily. Regarding its surface expression there exist conflicting results. Early studies demonstrated CD30L expression on subsets of resting and activated B and T cells as well as on mast cells, NK cells, eosinophils and neutrophils. These appeared at least partially not well controlled as the unspecific binding of antibodies was not prevented by antibody/CD30L competition experiments. More recent studies could not reproduce a surface expression of CD30L on isolated B cells, neutrophils, eosinophils or on primary mast cells (Kennedy et al., 2006). Apart from this discrepancy CD30L exerts pleiotropic activities and is particularly involved in cell proliferation and cell death (Cerutti et al., 2000; Rossi et al., 2001, 2002; Nishimura et al., 2005).

CD30 involvement in the physiological balance of Th1/Th2 response and tolerance induction

It is generally accepted (i) that signalling via CD30 promotes the development of Th2 cells and (ii) that CD30 represents a marker for Th2 cell populations. The expression of CD30 influences CD4$^+$ T cells to increase their production of Th2 cytokines (Rossi et al., 2001; Stanciu et al., 2001). Additionally, a CD30-mediated production of IL-13 independent of the T cell receptor (TCR) involvement was found in CD4$^+$ T cells (Harlin et al., 2002). Recently, CD30 has been demonstrated to be a marker of an immunomodulatory subpopulation of T cells, which regulates the balance between Th1 and Th2 type immune response (Pellegrini et al., 2003). Thus, CD30 plays an important role for the physiological balance between Th1/Th2 immune response. This hypothesis was further strengthened by the fact that CD30 was detected on a subpopulation of dendritic cells (DCs) known to modulate immune responses and tolerance (Pellegrini et al., 2005).

Determination of CD30/sCD30 in patients' samples

A prerequisite for the determination of CD30/sCD30 levels was the availability of CD30-specific monoclonal antibodies (mAb) which are reactive in Western blot, flow cytometry as well as in immunohistochemistry (IHC) on fresh frozen and paraffin-embedded tissue samples and/or peripheral blood mononuclear cells (PBMNCs). Indeed, there exists a number of CD30-specific mAbs, which have been successfully employed in different applications. In parallel to the monitoring of surface-bound CD30, several enzyme-linked immunosorbent assays (ELISA) have been developed for the detection of sCD30 (Josimov-Alasevic et al., 1989). The current sandwich-ELISA used by most researchers and commercially available (Bender MedSystems, Vienna, Austria or Biotest AG, Dreieich, Germany) is composed of two anti-sCD30 mAb for capturing and detecting the antigen. The detection antibody is used as a peroxidase conjugate.

Soluble CD30 as prognostic markers for lymphoma/leukaemia

CD30 is expressed on Hodgkin-Reed-Sternberg (H-RS) cells, anaplastic large cell lymphoma (ALCL) and adult T cell leukaemia/lymphoma (ATL) and represents a diagnostic marker of these malignancies (Nishioka et al., 2005). Since sCD30 is proteolytically cleaved from the surface of CD30$^+$ lymphoma cells, increased sCD30 serum levels have been established as a prognostic tool for the outcome of Hodgkin disease (Pizzolo et al., 1990; Gause et al., 1991; Nadali et al., 1994). Elevated serum levels of sCD30 (>100 U/ml) in the majority of Hodgkin disease patients were associated with a poor clinical outcome (Nadali et al., 1994). It is speculated that high sCD30 serum concentrations in patients reflect the tumour burden and block the biological effects of CD30L. In addition, the high frequency of EBV infection in Hodgkin disease and the fact that there exists an integration site of this virus close to the human CD30 locus also support the hypothesis that EBV infection might be responsible for the activated stage and the expression of CD30 in Hodgkin's cells (Hummel et al., 1992; Herbst et al., 1993).

CD30/sCD30 and other diseases

For several human autoimmune diseases such as rheumatoid arthritis Th2 cells are the main immune effector cells. In rheumatoid arthritis the CD30$^+$ T cells support the suppression of Th1 cells by a so far unknown mechanism thereby leading to declined numbers of Th1 cells (Gerli et al., 2000, 2001). In addition, increased sCD30 levels were found in synovial fluid (Gerli et al., 1995). Apart from rheumatoid arthritis an involvement of CD30$^+$ T cells has also been reported for systemic sclerosis (Mavalia et al., 1997) and atopic allergy (Bengtsson, 2001). In atopic dermatitis a correlation between the number of CD30$^+$ T cells and the levels of sCD30 was described (Folster-Holst et al., 2002). Elevated sCD30 serum concentrations have been correlated with disease activity/severity in patients with systemic lupus erythematosus (Caligaris-Cappio et al.,

1995), Wegner's granulomatosis (Wang et al., 1997) and HIV (Pizzolo et al., 1994, 1997). Increased concentrations of circulating sCD30 were also observed in patients with adult and juvenile atopic dermatitis (Yamamoto et al., 2000; Bengtsson, 2001), atopic asthma (Leonard et al., 1997a,b) and ulcerative colitis (Giacomelli et al., 1998), but there exist controversial results regarding an association with the disease progression in these settings.

Highly elevated sCD30 concentrations in hemodialysis patients

Elevated levels of sCD30 in the sera of patients on hemodialysis (HD) were found in several studies, suggesting that the increased sCD30 levels reflect the activation of Th2 lymphocytes and the consecutive induction of a Th2 response in the pathogenesis of patients undergoing HD. During the early phase of hemodialysis (first year) the CD30 serum concentrations increased to an average of about 130 U/ml and the Th1-specific immunity in comparison to the Th2 immunity was relatively depressed (Nakao et al., 2002). In the middle-term HD patients (1-10 years) the concentration of sCD30 decreased to values of about 95 U/ml, and again elevated in long-term patients (>10 years) to an average of 140 U/ml. These investigations point to significant time-dependent variations in the sCD30 concentrations during the disease course which most probably depend on the duration of the treatment. Hemodialysis generally may lead to a Th2 dominance, which might contribute to the well-known abnormalities in the immune system of these patients (Descamps-Latscha and Herbelin, 1993; Descamps-Latscha and Chatenoud, 1996). In the study of Nakao et al. (2002) the approximately three-fold increase in sCD30 found in hemodialysis patients (126±49 U/ml) was also found in patients suffering from chronic renal failure (CRF) (116±46 U/ml) in comparison to individuals of the healthy control group (49±18 U/ml). These data are in accordance with the results of Sengul et al. (2006) and our own data (Altermann et al., submitted) demonstrating that most of the potential renal graft recipients (about 90%) exhibit higher sCD30 serum levels (30-90 U/ml) than the healthy blood donors (0-30 U/ml). The elevated sCD30 levels are accompanied by an increased incidence of infection (Taskapan et al., 2000) and of different malignancies (Maisonneuve et al., 1999) in HD patients, suggesting that the increased sCD30 serum levels are involved in the impaired immunity of patients suffering from end-stage renal disease.

Hormone dependence of sCD30 levels

It has recently been suggested that hormones at least partially control the concentration of sCD30 (Barbano et al., 2001). In a comparative study sCD30 levels were significantly elevated both in children with CRF (~180 U/ml) and with primary deficiency of growth hormone/somatotrophin (GH) (~147 U/ml) when compared to a control group of healthy children (~10 U/ml). After treatment with recombinant GH an approximately 25% decrease of sCD30 plasma levels to 134 U/ml in patients with CRF or 35% decrease to 95 U/ml in patients with GH-deficiency, respectively, was observed. Based on these data it was concluded that there might exist an influence of GH on the sCD30 plasma level since patients' treatment with the recombinant hormone resulted in a down-regulation of sCD30. Thus, a direct influence of GH, which in uraemia is found at a reduced level on the T cell activation process, was proposed.

Increased sCD30 levels and kidney transplantation

In addition to diseases with dominating Th2 type immune responses in which elevated sCD30 serum levels are associated with an impaired course of disease (Caligaris-Cappio et al., 1995; Gerli et al., 1995; Wang et al., 1997; Bengtsson, 2001; Folster-Holst et al., 2002), the relevance of sCD30 in the context of allograft transplantation has been investigated and discussed. High pre-transplant serum levels of sCD30 appeared to be predictive for an increased risk of allograft rejection (Pelzl et al., 2002; Suesal et al., 2002). High post-transplant levels have been reported to be indicative for an ongoing acute kidney graft rejection (Roy et al., 2002; Slavcev et al., 2005; Dong et al., 2006). However, contradicting results concerning the clinical relevance of sCD30 as a pre-transplant parameter have been published, which will be extensively reviewed in this chapter. In addition, the high inter- and intra-individual variations of sCD30 levels in pre-transplantation sera demonstrated by our group (Altermann et al., submitted) challenge the feasibility of sCD30 as a predictor of allograft outcome.

Soluble CD30 - a predictive *pre-transplant* serum-derived marker for kidney graft survival/rejection?

The feasibility of sCD30 as a predictor of kidney graft outcome was first published by Pelzl and co-workers (2002) performing a retrospective study which comprised the pre-transplantation sera of 844 cadaver kidney recipients from three German transplant centres. Multiple factors, such as current panel reactive antibodies (PRA) demonstrating the degree of presensitisation, HLA-mismatches, immunosuppressive treatment and previous blood transfusions were considered in the multivariate analysis as co-variables. The two-year kidney graft survival of 68±6% was the lowest in recipients with a high sCD30 serum content (>800 U/ml) followed by recipients with an intermediate 2 year graft survival of 77±3% (400-800 U/ml). The highest survival rate of 86±1% was observed in recipients exhibiting sCD30 serum levels <400 U/ml. Increased risk ratios of 1.95 and 3.5 were shown for

patients with intermediate or high sCD30 levels, respectively. Even in selected, non-sensitised patients (PRA ≤ 5%) similar survival rates were obtained for the three groups. Based on this study, the determination of sCD30 levels in serum as a parameter to identify patients without evidence of PRA as patients of an increased or low rejection risk represented a suitable monitoring tool and thus appeared to be of clinical relevance. Prophylactic treatment with anti-lymphocyte antibodies as an induction therapy significantly increased the graft survival in "high CD30" patients from 58±10% to 74±7% suggesting that patients with high sCD30 serum levels should generally obtain prophylactic treatment with anti-T cell antibodies. In addition, CD30^{+} T cells were demonstrated to produce high amounts of the Th2 cytokine IL-10, thereby suppressing the Th1-type immune responses (Gerli et al., 2000, 2001). In this context patients with high IL-10 levels had been shown to exhibit an impaired kidney graft outcome and increased levels of sCD30 had been accompanied by high IL-10 levels (Weimer et al., 1996).

However, there exists a significant discrepancy in the quantity of pre-transplantation sCD30 levels in renal graft patients. Pelzl and co-workers (2002) defined the (i) "low risk group" with sCD30 levels <400 U/ml (599 recipients), (ii) the "intermediate group" with sCD30 levels of 400-800 U/ml (172 recipients) and (iii) "high risk group" with > 800 U/ml sCD30 (73 recipients). The latter contained 25 patients with sCD30 concentrations >1200 U/ml. So far, the reasons for these extremely high sCD30 values cannot be explained and have never been confirmed by subsequent investigations.

In a consecutive study of the same group (Suesal et al., 2002) pre-transplant sera of 3,899 cadaver kidney recipients provided by 29 transplantation centres from 15 countries were investigated for their sCD30 content. In this study the five-year graft survival rate of 901 recipients with high pre-transplant sCD30 levels (> 100 U/ml) was 64±2% and, thus, significantly lower when compared to 75±1% survival obtained from 2,998 recipients with a low sCD30 level (≤ 100 U/ml). The cut-off level between high and low serum levels was set at 100 U/ml based on multivariate Cox regression analysis and a serum content of >190 U/ml was regarded as exceptionally high. Furthermore, Suesal and co-workers (2002) provided evidence that the parameter low versus high sCD30 was detectable in first and regraft recipients, in presensitised and non-sensitised patients as well as in patients who received a highly matched kidney [0-1 HLA-mismatches (MM)] and a poorly matched kidney [4-6 MM]. The differences in the 5-year graft survival rates in all groups analysed were about 12-17%. An association between sCD30 serum levels and PRA was marginal, whereas the effects of both independent parameters on graft outcome were additive, resulting in a highly impaired outcome with a 5-year survival of only 55%. In contrast to the former study (Pelzl et al., 2002), no improved graft outcome in patients with high pre-transplant sCD30 levels after their

prophylactic treatment with anti-lymphocyte antibodies was indicated. The third study employing pre-transplant sera of 3,980 non-sensitised first cadaver kidney recipients provided by 33 transplantation centres from 17 countries demonstrated that in patients with low sCD30 serum concentrations (≤ 100 U/ml) the influence of HLA compatibility on the graft outcome was marginal, whereas it exhibited a significant effect in non-sensitised patients with high pre-transplant sCD30 levels > 100 U/ml (Suesal et al., 2003). In these recipients a statistically significant difference in the kidney survival rate between well (≤ 3 MM) and poorly (>3 MM) matched grafts of about 19% (71±2% versus 52±4%, respectively) was obtained. The data were exclusively derived from non-sensitised patients, since clinically relevant preformed antibodies (PRA >5% or ELISA-reactive antibodies) against HLA class I and/or HLA class II phenotypes were excluded. These results led to the conclusions that (i) especially non-sensitised, PRA-negative patients on the waiting list should be tested for serum sCD30 to allocate only well HLA-matched kidneys to high sCD30 patients and that (ii) sCD30 is a suitable prognostic marker comparable to PRA-determination (Suesal et al., 2003). The effects of the two parameters PRA and sCD30 level were described by the authors to be additive as shown by the graft survival curves for (i) low sCD30 (sCD30^{-})/ PRA>5% (PRA^{+}), (ii) sCD30^{-}/PRA<5% (PRA^{-}), (iii) high sCD30 (sCD30^{+})/ PRA^{-} and (iv) sCD30^{+}/PRA^{+} whereas indeed the combination of sCD30^{+}/PRA^{+} resulted rather in a synergistic positive graft outcome in comparison with the single parameters. The difference in the survival rate of well matched kidneys (MM ≤ 1) in sensitised and non-sensitised patients between low and high sCD30 patients was about 11% (Suesal et al., 2002) and 6% (Suesal et al., 2003), depending on the study, but the differences increased to 17% (Suesal et al., 2002) or 20% (Suesal et al., 2003) when outcomes of poorly matched kidneys of both studies were compared. The unexpectedly poor outcomes of both negative combinations sCD30^{+}/>3MM (only 52% survival versus calculated additive 66%) and sCD30^{+}/PRA^{+} (only 56% survival versus calculated additive 62%) in these investigations again showed that the effects of both combined parameters were rather synergistic than additive.

These results were more recently supported by the publication of Cinti et al. (2005), who attributed a higher impact to pre-transplant serum sCD30 than to PRA in the prediction of acute rejections within six month after kidney transplantation. All of the rejections observed in the study of Cinti and coworkers were objectively confirmed by biopsy. Using histopathological analyses of biopsies acute vascular rejections due to antibody-mediated cytotoxic mechanisms were also associated with higher pre-transplant sCD30 levels, whereas acute tubulointerstitial rejections were characterized rather by decreased levels of sCD30 which were even lower than in the healthy control group (Rajakariar et al., 2005).

The differences between these investigations and the former studies of Suesal and co-workers might be due to the coexistence of both cellular and humoral rejections in the patients. Although the number of patients investigated by Rajakariar and co-workers (n=67) and their distribution (51 with tubulointerstitial and 16 with the vascular rejection type) were not representative, their hypothesis is strengthened by the fact that in another study both acute cellular and humoral rejection grades coexisted in about 50% of the patients analysed who suffered from acute rejection (Mauiyyedi et al., 2002). The low sCD30 levels in patients with tubulointerstitial rejections might be due to the existence of CD8^{+} allospecific cytotoxic T cells which drive this type of rejection and which down–regulate Th2 cell responses (Chan et al., 1995). In the Th2-dominated humoral type of vascular reactions CD4^{+}/CD25^{+} regulatory T cells, which are positive for CD30, might suppress the cell-mediated rejection by inducing apoptosis of allospecific CD8^{+} T cells (Dai et al., 2004).

Soluble CD30 - a predictive *post-transplant* marker for kidney graft survival/rejection?

The use of sCD30 as a post-transplant indicator for an acute ongoing kidney graft rejection had first been published by Roy et al. (2002). The authors weekly monitored sera from 10 recipients for sCD30, IL-10 and TNF-α until two months post-transplantation. sCD30 levels were elevated after surgery until 2 weeks post-transplantation in 3/10 patients with an acute rejection, but not in non-rejecting patients. In addition, there was a correlation of the sCD30 levels with TNF-α, but not with IL-10 serum levels. Despite the low number of patients (n=10) monitored for sCD30 in combination with TNF-α this approach might be implemented for diagnosis of early acute rejections without an invasive biopsy. In addition, changes in the sCD30 levels of 65 kidney recipients without any recognizable infection 0 to 20 days post-transplantation was observed (Pelzl et al., 2003). The sCD30 plasma levels of the patients with an uncomplicated course (UC group, n=20) in comparison to those of the patients with acute tubular necrosis i.e. with a delayed start of renal function (ATN group, n=11) or with biopsy-proven acute rejections during the first 20 days (R group, n=25) significantly differed. Only patients of the ATN and UC groups showed a significant decrease in sCD30 during the first five days after transplantation. The pre-transplant sCD30 levels were reduced by 46% (UC group) and 25% (ATN group), but remained nearly unaltered in rejecting patients (R group). Thus, elevated sCD30 levels during the day 3 to 5 post-transplantation may be a predictor of graft rejection and allow the identification of rejecting patients 5 days earlier than the conventional approaches using biopsies. These data are in contrast to a report by Slavcev and co-workers (2005) demonstrating no significant pre-transplant differences in sCD30 levels between patients (n=117) undergoing allograft rejections

and non-rejecting patients. Both patients' groups, however, exhibited two weeks post-transplantation a decrease in the sCD30 concentration from an average of 104 U/ml to 31 U/ml for non-rejecting and to 63 U/ml for rejecting patients. Thus, the post-transplant level of sCD30 was more elevated in patients undergoing acute rejection. As determined by multifactorial analysis, elevated concentrations of sCD30 and anti-HLA class II antibodies were associated with an increased risk for acute rejection. Similar results were obtained by Dong et al. (2006), whose correlation between acute rejection and serum sCD30 levels after transplantation led to the classification into three distinct patients' groups. Although the pre-operative sCD30 levels did not significantly differ between the three groups (168-183 U/ml), the acute rejection (AR) group exhibited considerably higher sCD30 values (92±27 U/ml; n=49) on post-transplant day 5 than the uncomplicated course (UC) group (41±20 U/ml; n=171) or the delayed graft function (DGF) group (48±21 U/ml; n=11), respectively. The sCD30 levels on post-transplantation day 10 were virtually the same in all three groups. These data pointed to a time-dependence of post-transplantation sCD30 levels thereby further suggesting the sCD30 monitoring at day 5 post-transplantation as an adequate tool to identify patients with an impending acute graft rejection. In a recent study Sengul et al. (2006) confirmed the post-transplant decrease of the patients' sCD30 levels in comparison to their pre-transplant levels. The biopsy-proven rate of acute rejection was identified in 8 (AR+) out of 34 recipients (24%) who all exhibited pre-transplant sCD30 values >90 U/ml. 16 patients with serum sCD30 ≤ 90 U/ml did not show any case of acute rejection episode. However, it is noteworthy that the data of sCD30 pre-transplant levels exhibited extremely high standard deviations in that study [AR+ group: 195±116 U/ml (n=8) versus AR- group: 125±64 U/ml (n=42)]. Something similar holds for the sCD30 levels at post-transplant day 15, which were 53±33 U/ml (AR+) and 36±18 U/ml (AR-), respectively. In contrast, the values obtained at post-transplant day 30 did not exhibit significant alterations ranging between 47±20 U/ml in AR+ and 42±25 U/ml in AR- patients. Thus, the measurement of pre- and post-transplant sCD30 levels at day 15 may be an efficient method to identify kidney recipients with an increased risk of rejection. The application of several immunosuppressive drugs, including induction therapy, had no additional effect on post-transplantation sCD30 levels.

So far, there exists only one study determining the role of sCD30 as a predictor of a chronic renal transplant rejection (Weimer et al., 2005, 2006). Eighty-four renal allograft recipients (from 63 cadaver kidney donors and 21 living kidney donors) were monitored for both post-transplantation serum sCD30 and the monocyte/ macrophage activation marker neopterin as a risk marker for chronic allograft nephropathy. A chronic rejection within 2 years post-transplant significantly correlated with (i) increased sCD30 (> 60 U/ml) or (ii) neopterin

levels (> 40 nmol/ml) in serum samples obtained after 4 months and 12 months. The two year post-transplantation rejection rates were 40% (6/15) or 33% (5/15), respectively. Both risk factors in combination led to a rejection rate of 71% (5/7). However, the classical immunological risk factors like the HLA matching grade, the formation of PRA, pregnancies, blood transfusions and re-transplantations had no influence upon the sCD30 levels. In these investigations of Weimer et al. (2005, 2006) the pre-transplant sCD30 did not predict acute rejections (< 6 months) which is not in accordance with many other studies. Interestingly, cytomegalo virus (CMV) infections increased sCD30 levels within the first four post-transplantation months, but not after 12 months. Based on these results the influence of different immunosuppressive treatments on sCD30 levels was determined after one year. Tacrolimus-based immunosuppression (Azathioprine/Tacrolimus, Mycophenolate-Mofetil/Tacrolimus) in contrast to other combinatorial treatments analysed (Azathioprine/Cyclosporine, Mycophenolate-Mofetil/Cyclosporine) might represent the appropriate method for patients with increased pre-transplant sCD30 levels, since only this treatment modality resulted in a significant reduction of serum sCD30.

Prognostic pre- and post-transplant sCD30 values for the transplantation of organs other than kidneys

In contrast to the manifold studies concerning the impact of serum sCD30 on kidney allograft survival there exist only a few studies on the sCD30-dependent allograft outcome of other organs. In the study of Frisaldi et al. (2006) the genetic background was investigated by focusing on the analysis of a microsatellite region [(CCAT)n-sequence], which is located upstream from the CD30 promoter region, and known to be associated with sCD30 transcription levels (Croager et al., 2000; Kadin, 2000). No correlation between the low versus high expression microsatellite genotypes and the rate of rejections during the first year was found. Patients with genotypes including highly repeated alleles (putatively responsible for reduced CD30 levels) lacked significant variations in the survival of heart allografts. Although in comparison with the high numbers of kidney recipients only a limited number of heart recipients were investigated (n=83), low pre-transplant sCD30 levels appeared to be significantly associated with the survival of heart allografts. A one-year survival was found in 90% of patients with sCD30 levels ≤ 30 U/ml, but only 75% of patients with sCD30 levels exceeding 30 U/ml. A correlation between sCD30 (≤ 30 U/ml versus > 30 U/ml) and survival was also observed for long-term post-transplantation heart allografts (86% versus 75% at 2 years, 84% versus 73% at 3 years, 82% versus 67% at 4 years and 82% versus 63% at five years) indicating a predictive value of the pre-transplant sCD30 serum levels.

Concerning liver transplantation, the Th1 cytokine IFN-γ and sCD30 as an activation marker for Th2 cytokine-producing T cells were chosen to be monitored for a possible prediction of acute cellular rejection episodes in living donor liver graft recipients in the pre- and early post-operative periods (Kim et al., 2006). Pre- (day 0) and post-transplant sera on days 1, 3 and 7 from 32 living donor liver recipients after surgery were investigated for serum IFN-γ and sCD30. 14 recipients undergoing an acute rejection episode (AR+) exhibited significantly higher pre-transplant IFN-γ concentrations (74±23 pg/ml) than did recipients without an acute rejection episode (AR-; 10±2 pg/ml). On post-transplant days 1, 3 and 7 the serum IFN-γ levels were always higher in the AR+ group. The differences between both groups decreased during the first seven post-transplant days due to a significant reduction of serum IFN-γ in the AR+ recipients. In contrast to IFN-γ the pre-transplant sCD30 levels did not show significant differences. The average sCD30 level of the AR+ group exhibiting values of about 110 U/ml was even lower than that of the AR-group exhibiting levels of about 140 U/ml. Thus, the pre-transplant sCD30 serum levels in rejecting patients were not represented by higher values, which is in line with the studies of Slavcev et al. (2005) and Dong and co-workers (2006) monitoring kidney allograft recipients. The sCD30 levels of both groups decreased to about 60-70 U/ml without significant differences on days 1, 3 and 7, most probably due to the induced immunosuppressive treatment. The conclusion drawn by Kim et al. (2006) that the AR+ patients in contrast to the AR- patients showed a weak but non significant increase of their post-transplant sCD30 values (about 10%) between post-transplant day 1 and 3 is a puzzling interpretation of their raw data since the AR- group also exhibited a slight increase. Taken together, the data obtained by Kim and co-workers do not confer sCD30 a predictive clinical relevance for acute liver allograft rejection. Liver recipients in accordance with kidney recipients before transplantation displayed higher sCD30 values (93±58 U/ml) than healthy control persons (17±8 U/ml) indicating variations of the sCD30 concentrations during the disease course of liver failure similar to that observed during the hemodialysis treatment (Matinlauri et al., 2006). The mean pre-transplantation serum sCD30 value was marginally lower in non-rejecting (78±34 U/ml) versus rejecting (104±65 U/ml) patients. The post-transplant values of both groups after transplantation decreased clearly to 69±45 U/ml in non-rejecting and to 47±34 U/ml in rejecting patients. Thus, there existed an 18% higher reduction of sCD30 concentration in rejecting patients compared to non-rejecting patients. A correlation between serum sCD30 and anti-HLA class I antibodies or positivity in cross match assays was not observed by Matinlauri and co-workers. Concludingly, their investigation, in accordance with the data provided by Kim et al. (2006), showed that neither pre- nor post-transplant sCD30 levels were associated with acute rejection in liver allograft patients. All studies dealing with sCD30 as a *pre-* or *post-transplant* marker

mentioned in the text are summarized in table 1.

High variations in individual sCD30 levels in pre-transplantation patients over a year

In order to establish the measurement of serum sCD30 as a diagnostic tool for the patients of our kidney waiting list, we investigated whether there exist changes in the individual sCD30 pre-transplantation levels over time. In this study a quarterly monitoring of 652 patients was performed using a commercially available ELISA (Biotest AG, Dreieich, Germany) based on the assay originally developed by Josimov-Alasevic et al. (1989).

Calculations of the data were performed using the WINSTAT program. Serum samples of 203 healthy blood donors provided by the Department of Transfusion Medicine of the Martin Luther University (Halle, Germany) served as controls. In accordance with former publications (Barbano et al., 2001; Nakao et al., 2002; Slavcev et al., 2005; Matinlauri et al., 2006; Sengul et al., 2006) most of the putative recipients exhibited higher sCD30 values than healthy donors (0-30 U/ml) leading to mean values between 53 and 68 U/ml with high standard deviations (Table 2). The sCD30 levels of the 652 recipients did not show seasonal variations during the quarterly monitoring (Table 2). The frequency

Table 1. Summary of the studies on the feasibility of sCD30 serum levels as a pre- and / or post-transplant prognostic marker for allograft outcome of (A) kidney or (B) other solid organs.

A) kidney transplantations

Study / investigators (chronological order)	Organ	No. of recipients/ No. of centres	Feasibility as pre-transplant marker	Feasibility as post-transplant marker	sCD30 threshold values for risk groups
Pelzl et al., 2002 #	kidney	844 recipients/ German three centre study	**Yes**	not investigated	low risk group <400 U/ml middle risk group 400 to 800 U/ml high risk group >800 U/ml
Suesal et al., 2002 #	kidney	3,899 recipients/ 29 centre study from 15 countries	**Yes**	not investigated	low risk group <100 U/ml high risk group ≥ 100 U/ml
Roy et al., 2002	kidney	10 recipients	not investigated	**Yes**	elevated post-transplant sCD30 until 2 weeks
Suesal et al., 2003 #	kidney	3.980 recipients/ 33 centre study from 17 countries	**Yes**	not investigated	low risk group <100 U/ml high risk group ≥ 100 U/ml
Pelzl et al., 2003 #	kidney	65 recipients	not investigated	**Yes**	elevated post-transplant sCD30 at days 3 to 5
Cinti et al., 2005	kidney	24 recipients	**Yes**	not investigated	low risk group <100 U/ml high risk group ≥ 100 U/ml
Rajakariar et al., 2005	kidney	67 recipients	**Yes,** only for vascular rejection, but not for the tubulo-interstitial rejection type	not investigated	no threshold value recommended
Slavcec et al., 2005	kidney	117 recipients	**No**	**Yes**	elevated post-transplant sCD30 after 2 weeks
Dong et al., 2006	kidney	231 recipients	**No**	**Yes**	elevated post-transplant sCD30 at day 5 high risk group ≥ 65 U/ml
Sengul et al., 2006	kidney	50 recipients	**Yes**	**Yes**	high pre-transplant risk group ≥ 90 U/ml elevated post-transplant sCD30 at day 15
Weimer et al., 2006	kidney	84 recipients	not investigated	**Yes,** chronic rejection	elevated post-transplant sCD30 at 4 and 12 months

B) other solid organ transplantations

Study / investigators	Organ	No. of recipients	Feasibility as pre-transplant marker	Feasibility as post-transplant marker	sCD30 threshold values for risk groups
Frisaldi et al., 2006	heart	83 recipients	**Yes**	not investigated	high risk group >30 U/ml
Kim et al., 2006	liver	32 recipients	**No**	**No**	no threshold value recommended
Matinlauri et al., 2006	liver	54 recipients	**No**	**No**	no threshold value recommended

#: studies of the Dept. of Transplantation Immunology, Institute of Immunology, Medical School, University of Heidelberg, Germany.

of patients with sCD30 serum levels >100 U/ml was always lower during our quartal measurements than in the investigations published by Suesal and coworkers (2002, 2003). Interestingly, 109 of the 652 patients (16.7%) exceeded the borderline of 100 U/ml for one time to three times, whereas only 2.9% i.e. 19 of 652 patients showed sCD30 levels higher than the proposed "cut off" in all four quarterly measurements. The group of recipients with sCD30 levels permanently lower than 100 U/ml comprised 524 patients (80.4%). One hundred and fifty eight (24.2%) of 652 putative recipients exhibited sCD30 serum values lower than 30 U/ml for one time to three times indicating a certain rate of transient fluctuations down to sCD30 serum values of healthy control group members. Since in the studies performed so far pre-transplant sCD30 levels have only been determined once per individual, our data showed for the first time a high degree of variation in the individual sCD30 levels over time, which temporarily exceeded the proposed upper "cut off" at 100 U/ml, but also the lower "threshold level" of 30 U/ml between hemodialysis patients and healthy controls. Investigations regarding the variations of sCD30 over time are of particular importance, as in Middle Europe the waiting time of most recipients for the reception of a kidney graft is about three to four years, although many recipients exhibit waiting times ranging from five to ten years. During this period the patients may develop additional diseases based on the continued intoxication due to the lack of proper kidney function (Descamps-Latscha and Herbelin, 1993; Descamps-Latscha and Chatenoud, 1996; Taskapan et al., 2000; Nakao et al., 2002) suggesting that the variation of sCD30 serum levels may be caused by many reasons. Therefore, we hypothesize that sCD30 levels have to be determined over a time period in each individual of the transplantation list.

Conclusions

Although the data obtained by some investigators indicate a certain statistical impact of serum sCD30 levels the implementation of sCD30 levels as a pre-transplant or post-transplant marker can not be regarded as a breakthrough in the prediction of allograft rejection, since the data obtained by the various investigators are very heterogeneous or even contradictory as summarized in this article. The predictive value of serum sCD30 levels originally published by Pelzl et al. (2002) from a national three-centre-study and later from an international multicentre study (Suesal et al., 2002) was confirmed by Cinti et al. (2005), but highly narrowed by Rajakariar and co-workers (2005) who found a correlation between sCD30 levels and the corresponding rejection grades. In their study only the vascular rejection type was characterised by increased sCD30 pre-transplant levels, whereas the dominating tubulointerstitial type showed decreased sCD30 values even lower than those in the healthy control group. In addition, the use of sCD30 as a predictive marker could not be confirmed by the studies of Slavcev et al. (2005) and Dong et al. (2006). In both studies no significant differences in the sCD30 pre-transplant levels of rejecting and non-rejecting patients were observed. One can only speculate that the different grades of rejection and their possible co-existence which were detectable in nearly 50% of the patients suffering from acute rejection (Mauiyyedi et al., 2002) are not numerically reflected by the studies of Pelzl/Suesal and co-workers.

A general problem are the data evaluations which are nearly completely based on statistical calculations and which, additionally, are characterized by values with very high standard deviations but which do not investigate physiological aspects. Apart from the pivotal study of Dai et al. (2004) there exist, in addition to the multiple statistical analyses, only speculations about the origin, function and characteristics of CD30^{+} T cells as the origin of serum sCD30 and their possible influence on the phenomenon of allograft rejection. Therefore further studies clarifying these questions which also contribute to a better understanding of the pathophysiologic role of these cells in the rejection of an allograft are urgently required. Whether elevated sCD30 levels are genetically based or triggered by the patients' individual immunologic status as first discussed by Suesal and co-workers (2002) has not yet been the focus of investigations. Although a general genetic disposition with a minor impact can not be excluded (Frisaldi et al., 2006) the unexpectedly high individual time-dependent variations described in our study clearly favour the concept that mainly individual immunological events lead to the observed increase and decrease of serum

Table 2. No seasonal variation of mean sCD30 levels of 652 patients.

Date of serum collection	Mean value (MV)	Standard deviation (SD)	Expected border line
04 / 2005	52,52 U/ml	± 48,21	148,94
07 / 2005	67,25 U/ml	± 39,27	145,79
10 / 2005	58,47 U/ml	± 35,76	129,99
01 / 2006	68,19 U/ml	± 42,49	153,17

The sCD30 serum levels of 652 pre-transplant patients were quarterly monitored using an sCD30 ELISA. The expected border line is defined as MV + 2 SD.

sCD30 (Altermann et al., submitted) further arguing against one single measurement. Due to the heterogeneity of sCD30 levels its implementation as a pre-transplant marker cannot be justified for organ allocation to patients with sCD30 values higher than 100 U/ml.

Most of the investigations dealing with sCD30 as a predictive post-transplant marker demonstrate reduced serum sCD30 levels in kidney recipients without acute rejection episodes when compared to recipients with acute rejections. This reduction could not be observed in liver recipients (Kim et al., 2006; Matinlauri et al., 2006). However, different post-transplant days (5 days to 2 weeks) were employed for the determination of the sCD30 levels. According to the studies of Roy et al. (2002), Slavcev et al. (2005) and Sengul et al. (2006) a reduction of sCD30 levels had to be measured two weeks after the transplantation, whereas Pelzl et al. (2003) and Dong et al. (2006) detected a significant decrease in the patients' sCD30 levels without acute rejection episodes during the first five days. Therefore the measurement of sCD30 at the fifth post-transplant day was recommended. In addition, the parallel determination of some other parameters (e.g. cytokines or apoptotic parameters) might give further information and open a non-invasive strategy to predict acute rejections. The final aim of this strategy will be the avoidance of the highly invasive biopsy for which a substitution is urgently required. So far the value of post-transplant sCD30 levels for the prediction of acute rejections appears to be restricted to kidney transplantations since in the context of liver transplantations neither significant pre- nor post-transplant changes were observable (Kim et al., 2006; Matinlauri et al., 2006). Taken together, the high variations of sCD30 levels and their corresponding high standard deviations and variations of other statistical parameters as well as their time-dependence suggest the interpretation of individual sCD30 serum levels observed over time. Generalised threshold levels postulated cannot be applied to all patients. Further data on a larger panel of patients' samples with quarterly monitoring and in particular the association with other clinical parameters (e.g. infections, duration of hemodialysis) are a prerequisite to define sCD30 as putative biomarker for the prediction of kidney allograft rejections.

References

Agrawal B., Reddish M. and Longenecker B. (1996). CD30 expression on human CD8+ T cells isolated from peripheral blood lymphocytes from normal donors. J. Immunol. 157, 3229-3234.

Altermann W.W., Schlaf G., Rothhoff A. and Seliger B. (2007). High variation of individual sCD30 levels of pre-transplantation patients: sCD30 - a feasible marker for prediction of graft rejection? submitted for publication.

Barbano G., Cappa F., Prigione I., Pistoia V., Cohen A., Chiesa S., Gusmano R. and Perfumo F. (2001). Plasma levels of soluble CD30 are increased in children with chronic renal failure and with primary growth deficiency and decrease during treatment with recombinant human growth hormone. Nephrol. Dial. Transplant. 16, 1807-1813.

Bengtsson A. (2001). The role of CD30 in atopic disease. Allergy 56, 593-603.

Bengtsson A., Johansson C., Linder M.T., Hallden G., van der Ploeg I. and Scheynius A. (1995). Not only Th2 cells but also Th1 and Th0 cells express CD30 after activation. J. Leukoc. Biol. 58, 683-689.

Caligaris-Cappio F., Bertero M.T., Converso M., Stacchnini A., Vinante F., Romagnani F. and Pizzolo G. (1995). Circulating levels of soluble CD30, a marker of cells produce Th2-type cytokines, are increased in patients with systemic lupus erythematosus and correlate with disease activity. Clin. Exp. Rheumatol. 13, 339-343.

Cambiaggi A., Cantoni C., Marciano S., De Totero D., Pileri S., Tazzari P.L., Stein H. and Ferrini S. (1993). Cultured human NK cells express the Ki-1/CD30 antigen. Br. J. Haematol. 85, 270-276.

Cerutti A., Schaffer A., Goodwin R.G., Shah S., Zan H., Ely S. and Casali P. (2000). Engagement of CD153 (CD30 ligand) by CD30+ T cells inhibits class switch DNA recombination and antibody production in human IgD+ and IgM+ B cells. J. Immunol. 165, 786-794.

Chan S.Y., DeBruyne L.A., Goodmann R.E., Eichwald E.J. and Bishop D.K. (1995). In vivo depletion of CD8+ T cells results in Th2 cytokine production and alternate mechanisms of allograft rejection. Transplantation 8, 1155-1161.

Cinti P., Pretagostini R., Arpino A., Tamburro M.L., Mengasini S., Lattanzi R., De Simone P., Berloco P. and Molajoni E.R. (2005). Evaluation of pre-transplant immunologic status in kidney-transplant recipients by panel reactive antibody and soluble CD30 determinations. Transplantation 79, 599-601.

Croager E.J., Gout A.M. and Abraham L.J. (2000). Involvement of Sp1 and microsatellite repressor sequences in the transcriptional control of the human CD30 gene. Am. J. Pathol. 156, 1723-1731.

Dai Z., Li Q., Wang Y., Gao G., Diggs L.S., Tellides G. and Lakkis F.G. (2004). CD4+CD25+ regulatory T cells suppress allograft rejection mediated by memory CD8+ T cells via a CD30-dependent mechanism. J. Clin. Invest. 113, 310-317.

Del Prete G., De Carli M., Almerigogna F., Daniel C.K., D'Elios M.M., Zancuoghi G., Vinante F., Pizzolo G. and Romagnani S. (1995). Preferential expression of CD30 by human CD4+ T cells producing Th2-type cytokines. FASEB J. 9, 81-86.

Descamps-Latscha B. and Herbelin A. (1993). Long-term dialysis and cellular immunity: A critical survey. Kidney Int. 43, 135-142.

Descamps-Latscha B. and Chatenoud L. (1996). T cells and B cells in chronic renal failure. Semin. Nephrol. 16, 183-191.

Dong W., Shunliang Y., Weizhen W., Qinghua W., Zhangxin Z., Jianming T. and He W. (2006). Prediction of acute renal allograft rejection in early post-transplantation period by soluble CD30. Transpl. Immunol. 16, 41-45.

Duerkop H., Foss H.D., Eitelbach F., Anagnostopoulos I., Latza U., Pileri S. and Stein H. (2000). Expression of the CD30 antigen in non-lymphoid tissues and cells. J. Pathol. 190, 613-618.

Ellis T.M., Simms P.E., Slivnick D.J., Jack H. and Fisher R.I. (1993). CD30 is a signal-transducing molecule that defines a subset of human activated CD45RO+ T-cells. J. Immunol. 151, 2380-2389.

Folster-Holst R., Henseler T., Wehde J., Lemke H., Weichenthal M., Christophers E. and Hansen H.P. (2002). Soluble CD30 plasma concentrations correlate with disease activity in patients with atopic dermatitis. Acta Derm. Venereol. 82, 245-248.

Frisaldi E., Conca R., Magistroni P., Fasano M.E., Mazzola G., Patane F., Zingarelli E., Dall'Omo A.M., Brusco A. and Amoroso A. (2006). Prognostic values of soluble CD30 and CD30 gene polymorphisms in heart transplantation. Transplantation 81, 1153-1156.

Froese P., Lemke H., Gerdes J., Havsteen B., Schwarting R., Hansen H. and Stein H. (1987). Biochemical characterization and biosynthesis of the Ki-1 antigen in Hodgkin-derived and virus-transformed human B and T lymphoid cell lines. J. Immunol. 139, 2081-2087.

Gause A., Pohl C., Tschiersch A., De Costa L., Jung W., Diehl V., Hasenclever D. and Pfreundschuh M. (1991). Clinical significance of soluble CD 30 antigen in the sera of patients with untreated Hodgkin's disease. Blood 77, 1983-1988.

Gerli R., Muscat C., Bistoni O., Falini B., Tomassini B., Agea E., Tognellini R., Biagini P. and Bertotto A. (1995). High levels of the soluble form of CD 30 molecule in rheumatoid arthritis (RA) are expression of CD30+ T cell involvement in the inflamed joints. Clin. Exp. Immunol. 102, 547-550.

Gerli R., Pitzalis C., Bistoni O., Falini B., Costantini V., Russano A. and Lunardi C. (2000). CD30+ T cells in rheumatoid synovitis: mechanisms of recruitment and functional role. J. Immunol. 164, 4399-4407.

Gerli R., Lunardi C., Vinante F., Bistoni O., Pizzolo G. and Pitzalis C. (2001). Role of CD30+ T cells in rheumatoid arthritis: a counter-regulatory paradigm for Th1-driven diseases. Trends Immunol. 22, 72-77.

Giacomelli R., Passacantando A., Parzanese I., Vernia P., Klidara N., Cucinelli F., Lattanzio R., Santori, E. Cipriani P., Caprilli R. and Tonietti G. (1998). Serum levels of solubile CD30 are increased in ulcerative colitis (UC) but not in Crohn's disease (CD). Clin. Exp. Immunol. 111, 532-535.

Hansen H.P., Dietrich S., Kisseleva T., Mokros T., Mentlein R., Lange H.H., Murphy G. and Lemke H. (2000). CD30 shedding from karpas 299 lymphoma cells is mediated by TNF-alpha-converting enzyme. J. Immunol. 165, 6703-6709.

Harlin H., Podack E., Boothby M. and Alagre M.L. (2002). TCR-independent CD30 signaling selectively induces IL-13 production via a TNF receptor-associated factor/p38 mitogen-activated protein kinase-dependent mechanism. J. Immunol. 169, 2451-2460.

Herbst H., Stein H. and Niedobitek G. (1993). Epstein-Barr virus and CD30+ malignant lymphomas. Crit. Rev. Oncog. 4, 191-239.

Hummel M., Anagnostopoulos I., Dallenbach F., Korbjuhn P., Dimmler C. and Stein H. (1992). EBV infection patterns in Hodgkin's disease and normal lymphoid tissue: Expression and cellular localization of EBV gene products. Br. J. Haematol. 82, 689-694.

Josimovic-Alasevic O., Duerkop H., Schwarting R., Backe E., Stein H. and Diamantstein T. (1989). Ki-1 (CD30) antigen is released by Ki-1-positive tumor cells in vitro and in vivo. Partial characterization of soluble Ki-1 antigen and detection of the antigen in cell culture supernatants and in serum by an enzyme-linked immunosorbent assay. Eur. J. Immunol. 19, 157-162.

Kadin M.E. (2000). Regulation of CD30 antigen expression and its potential significance for human disease. Am. J. Pathol. 156, 1479-1484.

Kennedy M.K., Willis C.R. and Armitage R.J. (2006). Deciphering CD30 ligand biology and its role in humoral immunity. Immunology 118, 143-152.

Kim K.H., Oh E.J., Jung E.S., Park Y.J., Choi J.Y., Kim D.G., Lee K.Y. and Kang C.S. (2006). Evaluation of pre- and post-transplantation serum interferon-gamma and soluble CD30 for predicting liver allograft rejection. Transplant. Proceed. 38, 1429-1431.

Leonard C., Tormey V., Burke C. and Poulter L.W. (1997a). Allergen-induced cytokine production in atopic disease and its relationship to disease severity. Am. J. Respir. Cell. Mol. Biol. 17, 368-375.

Leonard C., Tormey V., Faul J., Burke C. and Poulter L.W. (1997b). Allergen-induced CD30 expression on T cells of atopic asthmatics. Clin. Exp. Allergy 27, 780-786.

Maisonneuve P., Agodoa L., Gellert, R., Stewart J.H., Buccianti G., Loewenfels A.B., Wolfe R.A., Jones E., Disney A.P.S., Briggs D., Mc Credie M. and Boyle P. (1999). Cancer in patients on dialysis for end-stage renal disease: An international collaborative study. Lancet 354, 93-99.

Matinlauri I., Hockerstedt K. and Isoniemi H. (2006). High serum soluble CD30 does not predict acute rejection in liver transplant patients. Transplant. Proc. 38, 3601-3604.

Mauiyyedi S., Crespo M., Collins A.B., Schneeberger E.E., Pascual M.A., Saidman S.L., Tolkhoff-Rubin N.E., Williams W.W., Delmonico F.L., Cosimi A.B. and Colvin R.B. (2002). Acute humoral rejection in kidney transplantation: Morphology, immunopathology, and pathologic classification. J. Am. Soc. Nephrol. 13, 779-787.

Mavalia C., Scaletti C., Romagnani P., Carossino A.M., Pignone A., Emmi L., Pupilli C., Pizzolo G., Maggi E. and Romagnani S. (1997). Type 2 helper T-cell predominance and high CD30 expression in systemic sclerosis. Am. J. Pathol. 151, 1751-1758.

Muta H., Boise L.H., Fang L. and Podack E.R. (2000). CD30 signals integrate expression of cytotoxic effector molecules, lymphocyte trafficking signals, and signals for proliferation and apoptosis. J. Immunol. 165, 5105-5111.

Nadali G., Vinante F., Ambrosetti A., Todeschini G., Veneri D., Zanotti R., Meneghini V., Ricetti M.M., Benedetti F., Vassanelli A., Perona G., Chilosi M., Menestrina F., Fiacchini M., Stein H. and Pizzolo G. (1994). Serum levels of soluble CD30 are elevated in the majority of untreated patients with Hodgkin's disease and correlate with clinical features and prognosis. J. Clin. Oncol. 12, 793-797.

Nakao K., Nagake Y., Okamoto A., Ichikawa H., Yamamura, M. and Makino H. (2002). Serum levels of soluble CD26 and CD30 in patients on hemodialysis. Nephron 91, 215-221.

Nawrocki J.F., Kirsten E.F. and Fisher R.I. (1988). Biochemical and structural properties of a Hodgkin's disease-related membrane protein. J. Immunol. 141, 672-680.

Nishimura H., Yajima T., Muta H., Podack E.R., Tani K. and Yoshikai Y. (2005). A novel role of CD30/CD30 ligand signaling in the generation of long-lived memory CD8+T cells. J. Immunol. 175, 4627-4634.

Nishioka C., Takemoto S., Kataoka S., Yamanaka S., Moriki T., Shoda M., Watanabe T. and Taguchi H. (2005). Serum level of soluble CD30 correlates with the aggressiveness of adult T-cell leukemia/lymphoma. Cancer Sci. 96, 810-815.

Pellegrini P., Berghella A.M., Contasta I. and Adorno D. (2003). CD30 antigen: not a physiological marker for Th2 cells but an important costimulator molecule in the regulation of the balance between Th1/Th2 response. Transpl. Immunol. 12, 49-61.

Pellegrini P., Totaro R., Contasta I., Berghella A.M., Carolei A. and Adorno D. (2005). CD30 antigen and multiple sclerosis: CD30, an important costimulatory molecule and marker of a regulatory subpopulation of dendritic cells, is involved in the maintenance of the physiological balance between TH1/TH2 immune responses and tolerance. The role of IFNbeta-1a in the treatment of multiple

sclerosis. Neuroimmunomodulation 12, 220-234.

Pelzl S., Opelz G., Wiesel M., Schnuelle P., Schoenemann C., Doehler B. and Suesal C. (2002). Soluble CD30 as a predictor of kidney graft outcome. Transplantation 73, 3-6.

Pelzl S., Opelz G., Daniel V., Wiesel M. and Suesal C. (2003). Evaluation of post-transplantation soluble CD30 for diagnosis of acute renal allograft rejection. Transplantation 75, 421-423.

Pizzolo G., Vinante F., Chilosi M., Dallenbach F., Josimovic-Alasevic O., Diamantstein T. and Stein H. (1990). Serum levels of soluble CD30 molecule (Ki-1 antigen) in Hodgkin's disease. Relationship with disease activity and clinical stage. Br. J. Haematol. 75, 282-284.

Pizzolo G. Vinante F., Morosato F., Nadali G., Chilosi M., Gandini G., Sinicco A., Raiteri R., Semenzato G and Stein H. (1994). High serum level of the soluble form of CD30 molecule in the early phase of HIV-1 infection as an independent predictor of progression to AIDS. AIDS 8, 741-745.

Pizzolo G., Vinante F., Nadali G., Krampera M., Morosato L., Chilosi M., Reiteri R. and Sinicco A. (1997). High serum level of soluble CD30 in acute primari HIV-1 infection. Clin. Exp. Immunol. 108, 251-253.

Rajakariar R., Jivanji N., Varagunam M., Rafiq M., Gupta A., Sheaff M., Sinnot P. and Yaqoob M.M. (2005). High pre-transplant soluble CD30 levels are predictive of the grade of rejection. Am. J. Transpl. 5, 1922-1925.

Rossi F.M., Degan M., Mazzocut-Zecchin L., Di Francia R., Aldinucci D., Pinto A. and Gattei V. (2001). CD30L up-regulates CD30 and IL-4 expression by T cells. FEBS Lett. 508, 418-422.

Rossi F.M., Degan M., Mazzocco F.T., Di Francia R., Aldinucci D., Paletto D., Vellenega E., Pinto A. and Gattei V. (2002). Co-expression of CD30 ligand and interleukin 4 (IL-4) receptors by acute myeloid leukaemia blasts is associated with the expansion of IL-4-producing CD30+ normal T cells. Br. J. Haematol. 117, 59-69.

Roy R., Dugre F.J., Berube L., Langlois S. and Lachance J.G. (2002). CD30 and TNF-alpha serum level as indicators of ongoing acute kidney graft rejection. Hum. Immunol. 63, 75 (abstract).

Schwab U., Stein H., Gerdes J., Lemke H., Kirchner H., Schaadt M. and Diehl V. (1982). Production of a monoclonal antibody specific for Hodgkin and Sternberg-Reed Cells of Hodgkin's disease and a subset of normal lymphoid cells. Nature 299, 65-67.

Sengul S., Keven K., Gormez U., Kutlay S., Erturk S. and Erbay B. (2006). Identification of patients at risk of acute rejection by pre-transplantation and post-transplantation monitoring of soluble CD30 levels in kidney transplantation. Transplantation 81, 1216-1219.

Shanebeck K., Maliszewski C., Kennedy M.K., Picha K.S., Smith C.A., Goodwin, R.G. and Grabstein K.H. (1995). Regulation of murine B cell growth and differentiation by CD30 ligand. Eur. J. Immunol. 25, 2147-2153.

Slavcev A., Lacha A., Honsova E., Sajdlova H., Lodererova A., Vitko S., Skibova J. and Striz I. (2005). Soluble CD 30 and HLA antibodies as potential risk factors for kidney transplant rejection. Transpl. Immunol. 14, 117-121.

Smith C.A., Gruss H.J., Davis T., Anderson D., Farrah T., Baker E., Sutherland G.R., Brannan C.I., Copeland N.G., Jenkins N.A., Grabstein K.H., Gliniak B., McAlister I.B., Fanslow W., Alderson M., Falk B., Gimpel S., Gillis S., Din W.S., Goodwin R.G. and Armitage R.J. (1993). CD30 antigen, a marker for Hodgkin's lymphoma, is a receptor whose ligand defines an emerging family of cytokines with homology to TNF. Cell 73, 1349-1360.

Smith C.A., Farrah T. and Goodwin R.G. (1994). The TNF receptor superfamily of cellular and viral proteins: activation, costimulation, and death. Cell 76, 959-962.

Stanciu L.A., Roberts K., Lau L.C., Coyle A.J. and Johnston S.L. (2001). Induction of type 2 activity in adult human CD8+ T cells by repeated stimulation and IL-4. Int. Immunol. 13, 341-348.

Stein H., Gerdes J., Schwab U., Lemke H., Mason D.Y., Ziegler A., Schienle W. and Diehl V. (1982). Identification of Hodgkin and Sternberg-Reed cells as a unique cell type derived from a newly-detected small cell population. Int. J. Cancer 30, 445-459.

Stein H., Mason D.Y., Gerdes, J., O'Connor N., Wainscoat J., Pallesen G., Gatter K., Falini B., Delsol G. and Lemke H. (1985). The expression of the Hodgkin's disease associated antigen Ki-1 in reactive and neoplastic lymphoid tissue: evidence that Reed-Sternberg cells and histiocytic malignancies are derived from activated lymphoid cells. Blood 66, 848-858.

Suesal C., Pelzl S., Doehler B. and Opelz G. (2002). Identification of highly responsive kidney transplant recipients using pre-transplant soluble CD30. J. Am. Soc. Nephrol. 13, 1650-1656.

Suesal C., Pelzl S. and Opelz G. (2003). Strong human leukocyte antigen matching effect in nonsensitized kidney recipients with high pre-transplant soluble CD30. Transplantation 76, 1231-1232.

Sun Y., Song, M. and Maeurer M.J. (2001). Segregation of effector mechanisms in a tumor-specific CD8+ T cell clone correlates with CD30 expression. Scand. J. Immunol. 54, 314-320.

Taskapan H., Utas C., Oymak F.S., Guelmez I. and Oezesmi M. (2000). The outcome of tuberculosis in patients on chronic hemodialysis. Clin. Nephrol. 54, 134-137.

Wang G., Hansen H., Tatsis E., Csernok E., Lemke H., Gross W.L. (1997). High plasma levels of the soluble form of CD30 activation molecule reflect disease activity in patients with Wegener's granulomatosis. Am. J. Med. 102, 517-523.

Weimer R., Zipperle S., Daniel V., Carl S., Staehler G. and Opelz G. (1996). Pre-transplant CD4 helper function and interleukin 10 response predict risk of acute kidney graft rejection. Transplantation 62, 1606-1614.

Weimer R., Suesal C., Yildiz S., Streller S., Pelzl S., Staak A., Renner F., Dietrich H., Daniel V., Feuring E., Kamali-Ernst S., Ernst W., Padberg W. and Opelz G. (2005). sCD30 and neopterin as risk factors of chronic renal transplant rejection: impact of cyclosporine A, tacrolimus, and mycophenolate mofetil. Transplant. Proc. 37, 1776-1778.

Weimer R., Suesal C., Yildiz S., Staak A., Pelzl S., Renner F., Dietrich H., Daniel V., Kamali-Ernst S., Ernst W., Padberg W. and Opelz G. (2006). Post-transplant sCD30 and neopterin as predictors of chronic allograft nephropathy: impact of different immunosuppressive regimens. Am. J. Transplant. 6, 1865-1874.

Yamamoto J., Adachi Y., Onoue Y., Kanegane H., Miyawaki T., Toyoda M., Seki T. and Morohashi M. (2000). CD30 expression on circulating memory CD4+ T cells as a Th2-dominated situation in patients with atopic dermatitis. Allergy 55, 1011-1018.