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Caner Süsal
Colette Dörr
Jürgen Groth
Gottfried May
Gerhard Opelz

# Pretransplant serum IgA concentration and IgA-anti-Fab autoantibody activity as prognostic indicators of kidney graft survival

C. Süsal (✉) · C. Dörr · G. Opelz
Department of Transplantation Immunology,
Institute of Immunology,
University of Heidelberg,
Im Neuenheimer Feld 305,
D-69120 Heidelberg, Germany

J. Groth · G. May
Department of Urology,
Friedrichshain Hospital,
Landsberger Allee 49,
D-10249 Berlin, Germany

**Abstract** IgA concentration and IgA-anti-Fab autoantibody activity were tested in pretransplant sera of 308 kidney graft recipients. Recipients with a serum IgA concentration of 2 g/l or greater had a 1-year graft survival rate of 83%, compared with a 68% rate in recipients with serum IgA of less than 2 g/l ($P < 0.005$). Serum IgA concentration and IgA-anti-Fab autoantibody activity were significantly associated ($r = 0.38$, $P < 0.0001$). Recipients with a high pretransplant IgA-anti-Fab activity had a significantly better graft survival rate (81%) than patients with low pretransplant IgA-anti-Fab (67%, $P < 0.025$). When IgA-anti-Fab and serum IgA were considered together, 137 recipients with high IgA-anti-Fab and high serum IgA had a 86% 1-year graft survival rate, which was significantly better than the 63% survival rate in patients with low IgA-anti-Fab and low serum IgA ($P < 0.0005$). The pretransplant serum IgA level and IgA-anti-Fab autoantibody activity were excellent predictors of kidney graft outcome.

**Key words** Serum IgA concentration · IgA-anti-Fab autoantibodies · Kidney graft survival · Immune network

## Introduction

We have shown previously that renal allograft recipients with high pretransplant IgG-anti-F $(ab')_2$ [1, 2] or IgA-anti-Fab autoantibody activity [3] have a significantly reduced rejection rate, and we have postulated that anti-Fab activity may be an important component of immune network regulation [4, 5]. Interestingly, Koka et al. have recently suggested a protective effect of IgA-anti-MHC class I antibodies in kidney graft recipients [6], which supports our contention that anti-Fab activity is directed against MHC-like variable regions of antigen receptor molecules, immunoglobulins (Ig) and T cell receptors (TcR) [4, 5]. Our recent work showing striking associations between IgA-anti-Fab autoantibody activity and disease progression in HIV-infected patients [7] and in patients with squamous cell carcinoma of the head and neck [8] provides further support for the immunosuppressive property of IgA-anti-Fab autoantibodies. Because of the known association of increased IgA serum immunoglobulins with HIV-1 infection [9], head and neck cancer [10], and IgA-anti-Fab autoantibody activity [8, 11], we deemed it of interest to investigate whether an increased serum IgA concentration itself reflects a suppressed immune status and whether the determinatio of serum IgA prior to transplantation may be predictiv of graft outcome in kidney transplant recipients.

## Materials and methods

### Patients and sera

At the Berlin Friedrichshain centre, 540 cadaveric kidney transplantations were performed between April 1986 and January 1991. Pretransplant sera of 474 recipients were sent to Heidelberg on dry ice and tested retrospectively for serum IgA content and serum IgA-anti-Fab autoantibody activity. Graft function was registered at 3, 6, and 12 months after transplantation. Of the 474 patients, 308 had a posttransplant follow-up after 1 year. Pretransplant sera were collected on the day of transplantation and kept frozen at $-20\,^{\circ}C$ until testing. Repeated thawing was avoided.

### Determination of serum IgA concentration

Serum IgA content (g/l) was determined in a commercial radial immunodiffusion assay (Behring, Marburg, Germany). Fifty-seven healthy controls had a mean serum IgA level of $2.1 \pm 0.1$ g/l.

### Determination of IgA-anti-Fab autoantibody activity (ELISA)

IgA-anti-Fab serum activity was determined in a specific ELISA assay. We coated 96-well microtiter plates (Nunc, Roskilde, Denmark) with 0.5 µg/ml of human IgG-Fab fragments (Dianova, Hamburg, Germany) at 37 °C for 16 h. The plates were washed and uncoated sites were blocked by incubation with 50 µl of 1% BSA-PBS solution at 37 °C for 3 h. We added 50 µl of 1:64 diluted test serum to the Fab-coated wells. PBS-Tween 0.05% was used as washing buffer and p-nitro-phenyl phosphate disodium solution (Sigma, St. Louis, USA) as substrate. Incubation steps with sera or antibodies were performed at 22 °C for 1 h. After each step the plates were washed four times with washing buffer. The reaction was developed with 50 µl of alkaline phosphatase conjugated goat antibodies specific for IgA-Fc (Dianova; working dilution 1:5000). Optical density was measured at 405 nm using a 340-ATTC microplate reader (SLT, Crailsheim, Germany).

### Statistical analysis

Statistical comparisons were performed by the chi-square test or regression analysis using the StatView SE + Graphics program (Microsoft, Abacus Concepts, Calif. USA).

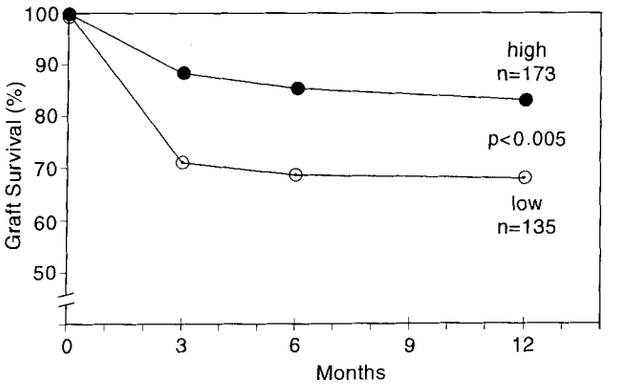


**Fig. 1** Kidney graft survifal in recipients with high ($\geq 2$ g/l) or low ($< 2$ g/l) pretransplant serum IgA concentration

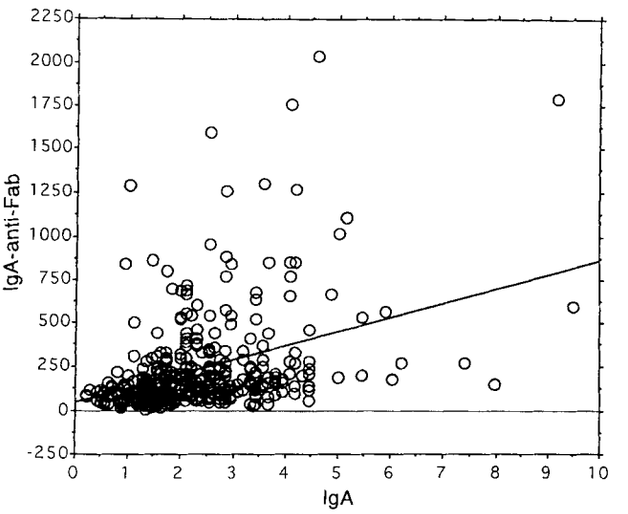


**Fig. 2** Association of serum IgA-anti-Fab autoantibody activity (optical density measured at 405 nm) with serum IgA concentration (g/l) in kidney graft recipients ($r = 0.387$, $P < 0.0001$). Note that some patients had high IgA-anti-Fab activity in spite of intermediate serum IgA concentrations

## Results

Kidney graft recipients were separated into two groups according to their pretransplant serum IgA concentration. Recipients with a serum IgA of 2 g/l or greater had a 1-year graft survival rate of 83%, which was significantly higher than the 68% graft survival rate in recipients with a serum IgA of less than 2 g/l ($P < 0.005$; Fig. 1). There was a significant association between serum IgA concentration and IgA-anti-Fab autoantibody activity ($r = 0.38$, $P < 0.0001$). However, as shown in Fig. 2, some patients with extremely high IgA-anti-Fab had an intermediate serum IgA concentration. In agreement with our previous report [3], kidney graft recipients with a pretransplant IgA-anti-Fab activity of 110 or greater (optical density measured at 405 nm) had a significantly better graft survival rate (81%) than patients with a pretransplant IgA-anti-Fab of less than 110 (67%), $P < 0.025$; Fig. 3). When IgA-anti-Fab activity was considered in combination with the serum IgA concentration, 137 recipients with IgA-anti-Fab of 110 or greater and serum IgA of 2 g/l or greater had a 1-year graft survival rate of 86%, significantly better than the 63% survival rate in the 62 patients with IgA-anti-Fab of less than 110 and serum IgA of less than 2 g/l ($P < 0.0005$; Fig. 4).

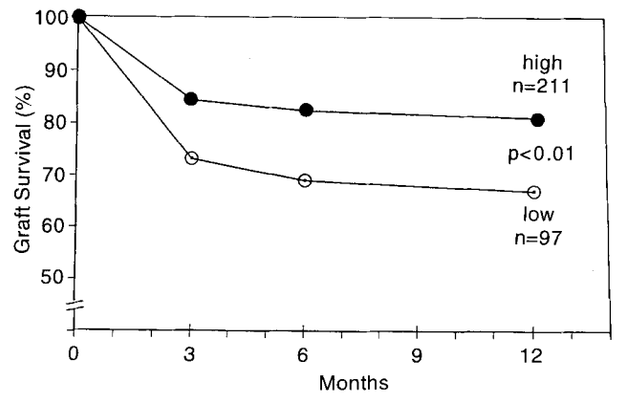


**Fig. 3** Kidney graft survival in recipients with high (optical density measured at 405 nm: $\geqq 110$) or low ($< 110$) pretransplant serum IgA-anti-Fab autoantibody activity

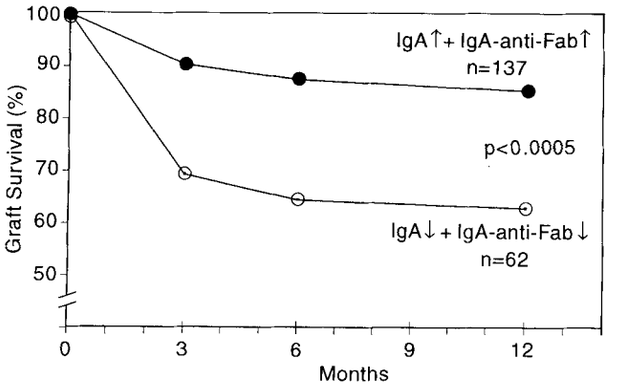


**Fig. 4** Kidney graft survival in recipients with high pretransplant serum IgA concentration (*IgA*↑) and high IgA-anti-Fab autoantibody activity (*IgA-anti-Fab*↑) or low IgA concentration (↓) and low IgA-anti-Fab (↓)

## Discussion

The present study demonstrated that determination of the serum IgA concentration prior to transplantation is a useful prognostic indicator of graft outcome in kidney transplantation. The predictive power was further improved by considering serum IgA concentration and IgA-anti-Fab autoantibody activity in combination. Patients with both a high serum IgA and a high IgA-anti-Fab had a strikingly better graft survival rate than patients with a low serum IgA and a low IgA-anti-Fab.

The significant association between serum IgA concentration and IgA-anti-Fab activity indicates that at least part of the increase was due to IgA-anti-Fab. The existence of IgA autoantibodies directed against the hinge region of immunoglobulin molecules may be involved, as IgA-anti-F $(ab')_2$ activity has been found to be associated with good kidney graft outcome in a previous study [3]. These findings may serve as an explanation for the improved survival rate of kidney grafts observed in patients with IgA nephropathy [12]. Elevated levels of IgA-IgG immune complexes and anti-F $(ab')_2$ antibodies have been reported in IgA nephropathy patients [13]. However, the original disease was diagnosed as IgA nephropathy in only one of the patients examined in the present study, indicating the existence of protective IgA antibodies in patients with diseases other than IgA nephropathy. IgA-anti-Fab autoantibody activity is associated with disease progression in HIV-infected patients [7] and in patients with squamous cell carcinoma of the head and neck [8]. Both diseases are associated with severe immune dysfunction, leading us to speculate that the appearance of IgA-anti-Fab antibodies in the circulation reflects a suppressed immune status.

We hypothesized that there may be a relationship between autoimmunity and viral infections due to cross-reactivity of viral antigens with Fab regions of immunoglobulin molecules [4, 5]. Sequence homologies exist between the CH1 region of IgG, Fab and the gp120 envelope protein of HIV-1 [4, 14]. Epstein-Barr virus (EBV) is believed to play an aetiological role in nasopharyngeal carcinoma as suggested by the occurrence of IgA antibodies against an epitope of EBV nuclear antigen [15]. The IgA-anti-Fab autoantibodies in dialysis patients may be the result of sequence homologies between human Fab molecules and antigenic structures on viruses such as EBV, CMV or alloimmunization by bloood transfusions [16].

It is generally accepted that helper T cells with weak complementarity to class II MHC molecules are positively selected in the thymus. It follows that TcR of most T helper cells have idiotypes that are weakly anti-MHC class II [17]. According to the immune network idea, TcR of some suppressor T cell clones and B cell antigen receptors (immunoglobulins) that interact with the anti-MHC class II like idiotypes of helper T cell clones would bear MHC class II like idiotypes. From investigations in HIV-infected patients, we have obtained evidence that anti-F $(ab')_2$ autoantibodies crossreact with a sequence of HIV-1 glycoprotein gp120 that resembles MHC class I and class II molecules. Our data and the recent study by Koka et al., showing that IgA-anti-MHC antibodies have a protective effect on kidney graft outcome [6], suggest the existence of antibodies directed against anti-MHC class II like idiotypes of helper T or MHC class II like idiotypes of suppressor T cell clones. Alloimmunization or virus infections may cause a destabilization of the immune

balance that is reflected in the dominance of anti-Fab in the circulation.

It is unlikely that the coappearance of increased serum IgA levels and immune dysfunction in diseases such as cancer and AIDS is accidental. The immune system is in a virgin state when antibodies of the IgM isotype are released into the circulation. Antibodies of the IgG isotype are effective weapons of the immune system that are produced in the immune state. Antibodies of the IgA isotype may reflect a terminated immune reaction and a suppressed immune state. The dominance of B cell clones that produce regulatory IgA antibodies appears to be an indicator of a tolerant immune system. Unlike cancer patients, transplant recipients may benefit from this phenomenon.

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## References


1. Süsal C, Guo Z, Terness P, Opelz G (1990) Role of anti-IgG autoantibodies in kidney transplantation. Immunol Lett 26:121–125
2. Süsal C, Groth J, Oberg HH, Terness P, May G, Opelz G (1992) The association of kidney graft outcome with pretransplant serum IgG-anti-F $(ab')_{2\tau}$ activity. Transplantation 54:632–635
3. Süsal C, Groth J, Tanzi-Fetta F, Kirste G, Dörr C, Terness P, May G, Stähler G, Opelz G (1992) Characterization of protective anti-Fab autoantibodies in kidney graft recipients. Transplant Proc 24:2523–2526
4. Süsal C, Kröpelin M, Daniel V, Opelz G (1993) Molecular mimicry between HIV-1 and antigen-receptor molecules: a clue to the pathogenesis of AIDS. Vox Sang 65:10–17
5. Süsal C, Hoffmann GW, Daniel V, Grant MD, Opelz G (1993) Complementarities and network interactions in AIDS. J Autoimmunity 6:601–610
6. Koka P, Chia D, Terasaki PI, Chan H, Chia J, Ozawa M, Lim E (1993) The role of IgA anti-HLA class I antibodies in kidney transplant survival. Transplantation 56:207–211
7. Süsal C, Daniel V, Dörr C, Zimmermann R, Huth-Kühne A, Opelz G (1993) IgA-anti-Fab autoantibodies and disease progression in AIDS. Immunol Lett 36:27–30
8. Süsal C, Maier H, Lorenz K, Opelz G (1994) Association of disease stage in head and neck cancer with IgA-anti-Fab autoantibodies. Int J Canc (in press)
9. Procaccia S, Lazzarin A, Colucci A, Gasparini A, Forcellini P, Lanzanova D, Foppa CU, Novati R, Zanussi C (1987) IgM, IgG and IgA rheumatoid factors and circulating immune complexes in patients with AIDS and AIDS-related complex with serological abnormalities. Clin Exp Immunol 67:236–244
10. Brown AM, Lally ET, Frankel A, Harwick R, Davis LW, Rominger CJ (1975) The association of the IgA levels of serum and whole saliva with the progression of oral cancer. Cancer 35:1154–1162
11. Süsal C, Oberg HH, Daniel V, Doerr C, Terness P, Huth-Kühne A, Zimmermann R, Opelz G (1994) Isotypes and IgG-subclasses of anti-Fab antibodies in HIV infected hemophilia patients. Vox Sang 66:37–45
12. Lim EC, Terasaki PI (1992) High survival rate of kidney transplants in IgA nephropathy patients. Clin Transplant 6:100–105
13. Schena FP, Pastore N, Ludovico R, Sinico RA, Benuzzi S, Montinaro V (1989) Increased serum levels of IgA1-IgG immune complexes and anti-F $(ab')_2$ antibodies in patients with primary IgA nephropathy. Clin Exp Immunol 77:15–20
14. Süsal C, Lewin IV, Stanworth DR, Terness P, Daniel V, Oberg HH, Huth-Kühne A, Zimmermann R, Opelz G (1992) Anti-IgG autoantibodies in HIV-infected hemophilia patients. Vox Sang 62:224–229
15. Foong YT, Cheng HM, Sam CK, Dillner J, Hinderer W, Prasad U (1990) Serum and salivary IgA antibodies against a defined epitope of the Epstein-Barr virus nuclear antigen (EBNA) are elevated in nasopharyngeal carcinoma. Int J Cancer 45:1061–1064
16. Süsal C, Guo Z, Terness P, Padanyi A, Petranyi G, Opelz G (1990) Induction of anti-F $(ab')_{2\tau}$ antibodies by buffy coat transfusions and their effect in kidney transplantation. Transplant Proc 22:1893–1984
17. Hoffmann GW, Kion TA, Grant MD (1991) An idiotypic network model for AIDS pathogenesis. Proc Natl Acad Sci USA 88:3060–3064