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Complement activation by anti-endothelial cell antibodies in MHC-mismatched and MHC-matched heart allograft rejection: Anti-MHC-, but not anti non-MHC alloantibodies are effective in complement activation

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Abstract Classical complement activation is a major effector mechanism in the development of vascular lesions contributing to allograft rejection. We investigated complement activation by alloantibodies reactive with graft endothelial cell (EC) alloantigens in settings of MHC-mismatched and MHC-matched (non-MHC-mismatched) rat heart transplantation (Tx). Allosera and heart allografts were collected at the day of rejection (day 7–8 and day 28–35 in MHC-mismatched and non-MHC-mismatched Tx respectively) or earlier. Allosera reactivity was studied in vitro using rat-heart-endothelial-cell (RHEC) lines expressing the appropriate donor MHC and non-MHC alloantigen profile. Immunohistochemical analysis of rejected heart allografts showed deposition of alloantibodies in both MHC-mismatched and MHC-matched heart allografts, but expression of C3 was only seen in the vasculature of MHC-mismatched grafts. FACS analysis showed that anti MHC as well as anti non-MHC allosera were reactive with donor EC cell surface antigens. Both sera had similar IgG subclass profiles of anti-endothelial cell antibodies. Complement activation by anti MHC and anti non-MHC alloantibodies on EC was measured by FACS analysis of C3 and C5b-9 (MAC) expression. Distinct expression of C3 was noticed for EC incubated with anti-MHC allosera, but hardly for EC incubated with anti non-MHC allosera. C5b-9 was low but showed no difference between the two allosera. However, complement-mediated cytotoxicity experiments showed that functional (lytic) MAC was induced with anti MHC allosera but hardly with anti non-MHC allosera. Our data show that in settings of MHC-matched heart transplantation alloantibodies against endothelial non-MHC alloantigens are generated, but, in contrast to alloantibodies to MHC alloantigens, these alloantibodies have only poor complement-activating and lytic potentials. Whether anti non-MHC allolantibodies effect other biological processes relevant for heart allograft vasculopathy, including development of graft arteriosclerosis, needs further elucidation.

Key words Alloantibodies · Complement · Heart endothelial cells · Transplantation · MHC · Non-MHC

Introduction

Despite progress in immunsuppressive strategies following transplantion (Tx), rejection of transplanted hearts is still a clinical problem. The endothelium of the graft vasculature is considered a major target tissue in the rejection process because of its alloantigen profile and the direct contact with the circulating immune reactants of the recipient. These immune reactants are of cellular and humoral (antibody) nature [8, 10, 30, 35] and may readily interact with constitutively expressed MHC class I and, in settings of immune reactivity, induced MHC class II antigens on the EC of the graft vasculature.

The role of antibodies in allograft rejection is controversial (except for hyperacute rejection) due to the regular absence of alloantibodies in rejected grafts. However, although cell-mediated immune mechanisms are considered to be the main effector mechanisms in rejection, the contribution of humoral mechanisms in allograft rejection has been shown [9, 16]. Particularly in chronic rejection, a more dominant role for alloantibodies in the development of vascular lesions is expected [2, 6, 31, 32, 36]. Among the mechanisms effecting antibody-mediated vascular damage, complement activation is a major one. For instance, complement activation may lead to formation of the membrane attack complex (MAC or C5b-9) and subsequent EC lysis. In addition, it leads to the formation of split products of early complement components such as the anaphylatoxins C3a and C5a which influence recruitment and effector functions of platelets and leukocytes [3]. It has also been shown that classical complement activation through antibody reactivity with EC triggers induction of adhesion molecules such as ICAM-1, VCAM-1 and E-selectin, apparently through autocrine stimulation by induced endothelial IL-1 [7, 25].

While MHC molecules can be considered the most dominant antigens in alloantibody reactivity with graft EC, a series of studies have demonstrated that also non-MHC alloantigens are involved in rejection processes [1]. Such alloantibody reactivity with endothelial non-MHC alloantigens appears associated with vasculopathy, and in particular with graft arteriosclerotic lesions [11, 19].

In the present study we investigated the potentials of alloantibodies to endothelial MHC versus non-MHC alloantigens in complement activation. An *in vivo* rat heart allograft rejection model in combination with an *in vitro* culture system of donor-representative heart EC was used [15]. Allosera from BN recipients of an allogeneic (LEW) or congenic (LEW.1 N) heart, respectively, were tested for reactivity and complement activation with cultured heart EC expressing the appropriate donor MHC or non-MHC alloantigen profile. In addition, alloantibody reactivity with graft endothelium and complement activation was immunohistochemically studied in situ in tissue sections of rejected heart allografts.

We show here that in heart allograft rejection alloantibodies reactive with endothelial MHC as well as with non-MHC alloantigens are generated. Significant complement activation with lytic potentials is seen when alloantibodies are reactive with endothelial MHC class I as well as with class II alloantigens. However, although a distinct alloantibody reactivity with endothelial non-MHC alloantigens was shown, only minor complement activation by these alloantibodies was measured. The results are discussed in the context of development of graft vasculopathy in heart allograft rejection.

Materials and methods

Animals

Lewis, (LEW; RT.1^l), Brown Norway (BN; RT.1^n) and congenic Lewis 1 N (LEW.1 N, RT. 1^n) rats were obtained from the Central Animal Facility of the University of Maastricht. Male rats at the age of 8–12 weeks were used. Animals were bred under specified pathogen free conditions and had free access to food and water. Animal experiments were carried out according the Dutch law on Animal experiments. Prior to the experiment, permission was obtained from the Ethical Committee on Animal Experimentation of the University of Maastricht under number 9925.

Heart transplantation and material collection

Hearts were routinely transplanted heterotopically in the abdomen. In short: the donor heart aorta and artery pulmonalis were end-to-side anastomized to the recipient aorta abdominalis and vena cava inferior, respectively [15, 33]. Transplantations were considered technically successful when heart beat was more than 250 beats/min on the second day after transplantation (Tx). Transplanted hearts were considered rejected when heart beat was absent or less than 100/min. The following heart Tx were executed:

1: LEW → BN, (MHC and non-MHC) -mismatched;

bloodsamples and tissues were collected at the day of rejection (7–8 days after Tx).

2: LEW.1 N → BN, non-MHC mismatched; bloodsamples and tissues were collected at day 8, 15, 21 days after Tx, and the day of rejection (29–35 days after Tx).

3: BN → BN, matched non rejected hearts; bloodsamples and tissues were collected at day 8 and 29 after Tx.

Sera were prepared from bloodsamples and stored at –70 °C until use. Unless mentioned otherwise, used sera were from the day of rejection.

Endothelial cells

For in vitro EC studies 3 EC lines from LEW heart origin were used. These Rat Heart Endothelial Cell lines RHEC 3, RHEC 10, and RHEC 11 were prepared as previously described [14]. EC were routinely cultured in EC culture medium (40% RPMI 1640 (Gibco BRL, Life Technologies B.V., Breda, the Netherlands, 40% M199 (Gibco BRL), 20% inactivated FCS (Integro, Bel-

gium), 0.5 µl/ml bovine brain extract (14.93 mg/ml) prepared as described by Maciag [26], 13 mM $NaHCO_3$, 50 U/ml penicillin, 50 µg/ml streptomycin, 2 mg/ml Fungizone, 10 mM Hepes, 20 U/ml heparin and 2 mM L-Glutamin). Cells were cultured using 5% CO_2 in air, at 37 °C. For passaging the cells, confluent monolayers were treated with trypsin (0.25%)/ EDTA (0.2%) in phosphate-buffered saline (PBS) for 1 min. After detachment, trypsin was neutralized with EC Work medium [(90% M199 (Gibco BRL), 10% inactivated FCS, 50 U/ml penicillin, 50 µg/ml streptomycin and 2 mM L-Glutamin and 25 mM Hepes)].

Cytokine stimulation of RHEC cultures

Confluent EC monolayers were stimulated with a combination of the cytokines TNFα and IFNγ for 24–72 h, as previously described [13]. Recombinant human TNFα (a gift from Prof. Dr. W. Fiers, Gent, Belgium) was used in a 400 U/ml concentration. Recombinant rat IFNγ (a gift from Dr. P. van de Meide, TNO Rijswijk, The Netherlands) was used in a 200 U/ml concentration.

Detection of allosera reactivity with EC

Suspensions of EC from trypsinized monolayers were used (see above). 5×10^5 cells were incubated with 5 × diluted heat-inactivated allosera in PBS containing 0.5% bovine serum albumin (PBSA) for 1 h, on ice. Cells were washed 3 times with PBSA and incubated with saturating amounts of a FITC conjugated second step swine $F(ab)_2$ anti rat Ab (DAKO, Denmark) for 30 min, on ice. After washing 3 times, cells were analyzed by flow cytometry, using a FACS-sort (Becton Dickinson, Mountain View, CA, USA). Data were collected from 1×10^4 cells. For data analysis the program Cell Quest was used (Becton Dickinson, Mountain View, CA, USA).

Isotyping of the anti-endothelial antibodies in allosera

EC (5×10^5), were incubated with allosera in a 5x dilution for 1 h, 0 °C. Cells were washed 3 times with PBSA and incubated with polyclonal rabbit anti-rat IgM, IgG1, IgG2a, IgG2b, IgG2c (a generous gift from Prof. H. Bazin, VCL, Louvain, Belgium), for 1 h on ice. Cells were washed 3 times with PBSA and incubated with FITC labelled swine $F(ab)_2$ anti rabbit Ig (1/200, DAKO, Denmark) for 30 min, on ice. Cells were washed 3 times with PBSA and analyzed by flow cytometry (see above).

Detection of complement activation by the alloantisera

Cytokine stimulated EC ($5 \times 10^{5)}$ were incubated for 1 h, 0 °C with the allosera from the different Tx models. Allosera were 5x diluted in 0.5% PBSA. Rat or GP complement was added simultaneously (v/v/v: 1/1/1). Cells were washed 3 times with PBSA and incubated with directly FITC-labelled polyclonal rabbit anti-rat C3 prepared in our laboratory, monoclonal 2A1, a mAb against rat C5b-9 (a kind gift of W.G. Couser, Seattle, USA) or with polyclonal rabbit IgG anti GP C3 (1/100) (Kindly donated by M. Daha, Leiden, the Netherlands), for 1 h, 0 °C. Cells were washed 3 times with PBSA and, in case of two step staining, incubated for 30 min with FITC-conjugated swine $F(ab)_2$ anti rabbit Ig (1/200, DAKO, Denmark) or FITC-conjugated goat anti mouse IgG, (1/100, Cappel, The Netherlands) . Cells were washed 3 times with PBSA and analyzed by flow cytometry (see above).

(Immuno)histochemistry

Collected hearts were cut and routinely processed for paraffin and frozen tissues. Paraffin tissues were routinely sectioned and hematoxylin/eosin-stained. Frozen material was used for sectioning 6 µm thick sections that were stained using the 2-step immunoperoxidase technique [17]. In brief: Sections were aceton-fixed and blocked for endogenous peroxidase reactivity by preincubation with 0.3% H_2O_2 in PBS for 10 min. First step antibodies were diluted to pre-defined saturated concentration in 0.05% PBSA, and incubated under humidified conditions for 1 h at room temperature. After washing in PBS, incubation with the appropriate peroxidase-labeled second step antibodies in PBSA containing 3% normal rat serum was carried out for 30 min. After washing, sections were incubated with a diaminobenzidine solution containing 0.02% H2O2 for 10 min and subsequently rinsed with tap water.

Antibodies:

- Rabbit anti-rat C3 (donated by Dr. M. Daha; Leiden, The Netherlands)
- mAb 2A1, Mouse anti-rat C5b-9 (donated by Dr. W. Couser; Seattle, USA)
- Rabbit anti-rat IgM, anti-rat IgG1, anti-rat IgG2a, anti-rat IgG2b, anti-rat IgG2c (donated by Dr. H. Bazin; Leuven, Belgium)
- Rabbit F(ab)2 anti-mouse IgG/peroxidase-labelledP (Dako, Denmark)
- Swine F(ab)2 anti-rabbit IgG/peroxidase-labelledP (Dako, Denmark)

After immunostaining, sections were counterstained with hematoxylin, dehydrated and coverslipped with Entallan (Merck, Germany).

Complement-mediated cytotoxicity of alloantisera

Cytotoxicity against EC was determined using a ^{51}Cr release assay. Endothelial cell suspensions were obtained as mentioned above. Suspensions of 5×10^7 EC/ml were incubated with 100 µCi ^{51}Cr / ml (Amersham International, England) in EC culture medium for 45 min at 37 °C. During labeling, the cells were permanently rotated. The labelled EC suspensions were washed twice with EC Work medium and per well, 1×10^5 cells in 25 µl were incubated with 25 µl 5 x diluted heat-inactivated allosera in EC culture medium, and 25 µl agarose-adsorbed guinea pig (GP) or full rat serum as complement source, for 60 min at 37 °C. In all experiments, hyper immune serum obtained after immunization of BN rats with LEW LNC was included as a standard for maximal biological release. Hyper immune serum was collected 3 days after a series of 3 immunizations (2 week intervals) with LNC. Negative controls included were control Tx sera. All experiments were performed in triplicate with the 3 endothelial cell lines. ^{51}Cr release was measured in a gamma counter. Percentages specific lysis were calculated as follows: % specific release = [(cpm alloserum – cpm control Tx serum) / (cpm immune serum – cpm control Tx serum)] × 100.

Results

Deposition of alloantibodies in the vasculature of MHC-mismatched and of MHC-matched heart allografts

Tissues of rejected heart allografts and non-rejected syngeneic heart grafts were examined for antibody reactivity with the graft vascular endothelium. Both in MHC-mismatched and in MHC-matched (non-MHC mismatched) rejected allografts, not, however, in syngeneic grafts, deposition of antibodies along the entire graft vasculature was observed, demonstrating the production of antibodies reactive with MHC and non-MHC alloantigens respectively (Fig. 1A, C, D). Although this is no definite proof of specific antibody reactivity with the EC of the vasculature, the luminal staining pattern supported it. By using isotype specific antibodies, it was shown that the alloantibodies were of the IgM- and of the various IgG subclass types. In the non-MHC-mismatched Tx setting allografts were also examined before rejection and showed alloantibodies of the various IgG subclass types present already at day 8 after Tx. (see also Fig. 1E). Also, IgM alloantibodies were noticed at day 8 after Tx, but their levels declined in the subsequent period.

Specific reactivity of anti MHC and anti non-MHC allosera with heart EC cultures

To certify that in situ observed alloantibody reactivity in the graft vasculature was with graft endothelial cell alloantigens, it was studied whether anti MHC and anti non-MHC allosera recognized EC in cultures of the 3 RHEC lines, which express the appropriate donor MHC and non-MHC haplotype . Immunostaining showed that anti MHC allosera were reactive with all 3 RHEC lines. Allosera reactivity was increased (higher mean staining intensity) when cytokine-stimulated EC were used which, as previously shown [13], expressed in addition to MHC class I antigens also class II antigens. Antibody isotyping showed that both IgM and the various IgG subclasses contributed to allosera reactivity with endothelial MHC alloantigens. Anti non-MHC allosera, collected at days 8, 15, 21 and at the day of rejection, showed reactivity with EC of all 3 RHEC lines. Reactivity did not increase when cytokine-stimulated EC were used (Table 1). Isotyping showed that anti non-MHC alloantibody reactivity with EC was mainly confined to antibodies of the various IgG subclasses. Control Tx sera did not react with either non-stimulated or cytokine-stimulated EC.

Table 1 Anti MHC and anti non-MHC allosera are reactive with donor heart EC. Reactivity of the allosera was measured by immunostaining of EC from all three RHEC lines. Stimulation of EC was executed with the cytokines TNFα + IFNγ for 24–48 h. – : all cells negative; + : all cells positive; + + : increased staining intensity

Allosera	Non-stimulated EC	Cytokine-stimulated
Control Tx	–	–
Anti MHC	+	+ +
Anti non-MHC	+	+

Complement C3 binding on EC is effected by anti MHC, however hardly by anti non-MHC alloantibodies

Complement activation on graft EC by anti MHC and anti non-MHC allosera was tested in vitro using cytokine-stimulated RHEC lines by measuring C3 and C5b-9 (MAC) binding. Allosera-induced binding of these complement factors was tested and compared with immune serum and control Tx sera. FACS analysis (Fig. 2) showed that immune serum and anti MHC allosera, incubated either with GP or rat complement, induced C3 formation on EC. Anti non-MHC allosera showed some C3 expression with GP complement but hardly with rat complement. Effects of allosera on formation of C5b-9 complexes on EC was studied with rat complement due to lack of antibodies to the GP C5b-9 complex. Low C5b-9 expression was measured in EC treated with either immune serum, anti MHC, or anti non-MHC allosera. In this respect for anti-non-MHC allosera (but not for anti MHC and immune serum), C5b-9 binding as a parameter for complement activation did not correspond with the data as collected for rat C3.

Complement-mediated cytotoxicity for EC is induced by anti MHC but hardly by anti non-MHC allosera

Whether allosera-induced complement activation on EC as measured by FACS analysis for C3 and C5b-9 binding has lytic effects was tested in a 51 chromium release assay using cytokine-stimulated RHEC cultures. As shown in Fig. 3, a distinct lytic effect was measured with anti MHC allosera using GP serum as source for complement. With anti-non-MHC allosera only minor complement-mediated cytotoxicity was measured using GP complement. When in the cytotoxicity assay, the GP complement source in the allosera incubations was exchanged with rat complement, a much lower lytic effect was observed for both sera (Fig. 3). Since also the lytic effect of immune serum was much lower with rat versus GP serum, it was thought that this could be related to the inhibitory activity of syngeneic complement controlling proteins (CCP) in EC. To obtain information about this, both complement sources were tested for cytotoxic effects on sheep (xenogeneic) red blood cells (SRBC) coat-

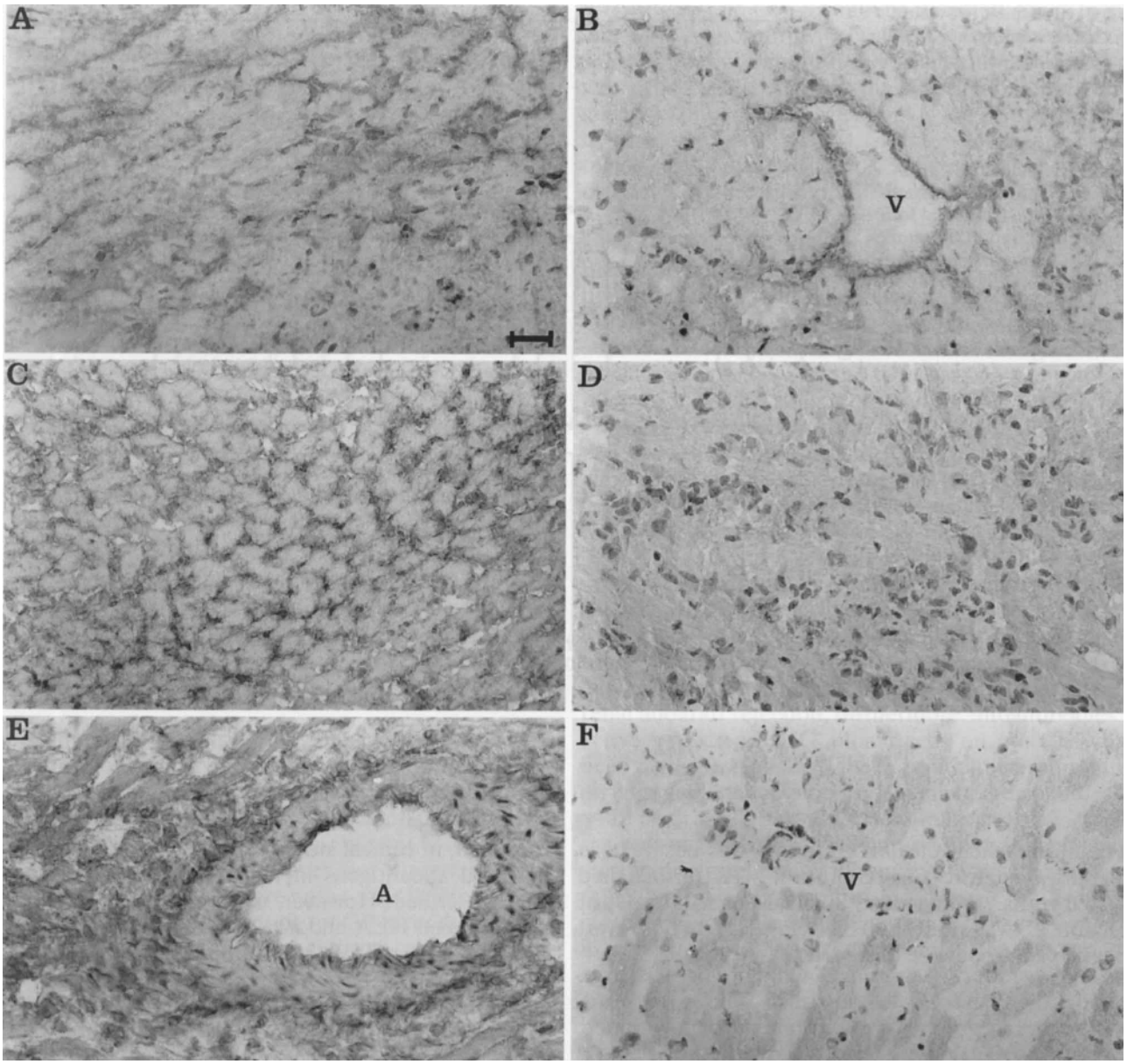

Fig. 1 A–F Immunoperoxidase staining on frozen sections of heart allografts. *Upper 2 panels* Immunostaining of a rejected MHC-mismatched heart. **A** Staining with anti-IgG2a showing deposition of alloantibody in the myocardial capillary network. **B** Anti-C3 staining shows that C3 is present in the vasculature. Note the stained venular vessel (V). *Middle 2 panels* Immunostaining of a rejected MHC-matched heart. **C** Ig2a deposition in the capillary network. **D** Anti-C3 staining showing the nearly absence of C3 in the graft vasculature. *Lower 2 panels* **E** Anti-IgG2a staining of a MHC-matched allograft 8 days after Tx showing the presence of IgG2a in and around a large artery (*A*). **F** Negative control staining of a rejected heart allograft. Note the negative venular vessel (*V*). *Bar* measures 10 μm

ed with anti-SRBC Ab. In this setting, in which a role for CCP is excluded, GP complement also showed an about five times higher activity than rat complement. This indicates that the low effects of rat complement in cytotoxicity assays with anti MHC and anti non-MHC allosera is likely not an effect of CCP activity in EC.

In situ deposition of complement factors in the allograft vasculature

To obtain more information about allosera-induced complement activation on graft EC, we studied C3 and C5b-9 deposition in tissue sections of allografts. In

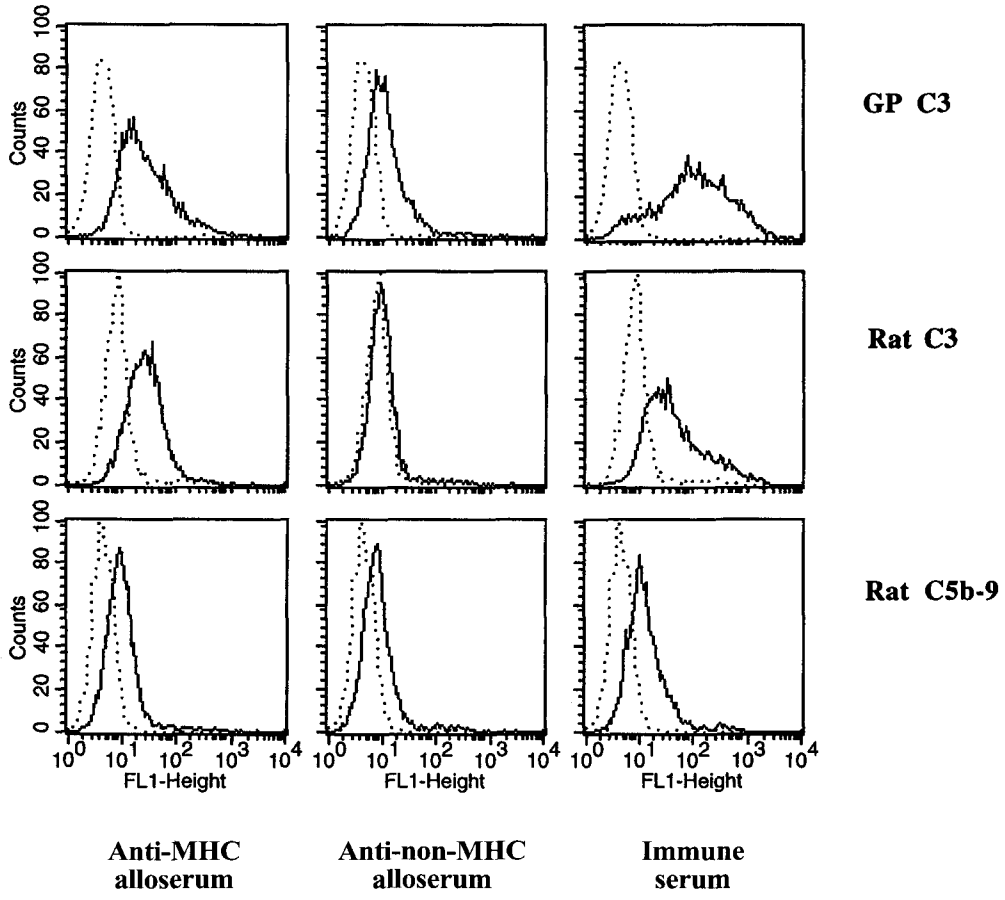

Fig. 2 Complement activation on EC is effected by anti-MHC but not much by anti non-MHC allosera. Immunofluorescence staining for C3 and C5b-9 (MAC) expression was performed with TNFα + IFNγ (24–48 h)-stimulated EC from RHEC 3. For complement activation, EC were incubated for 1 h with either anti MHC, anti non-MHC allosera, or immune serum in the presence of rat or GP complement. Binding of the complement factors by the test sera was compared with those from control Tx sera using the Kolgomorow-Smirnov test. Sera significantly induced the binding of the complement factors ($P < 0.001$), except for Rat C3 induction by the anti non-MHC alloserum. ··· Control Tx sera, — test sera

MHC mismatched rejected grafts, C3 was observed in the graft vasculature (Fig. 1B). In non-MHC mismatched rejected grafts, studied at day 8 after Tx and at the day of rejection, C3 was hardly detected (Fig. 1D). In both graft types no C5b-9 could be observed.

Discussion

We have been studying complement activation by anti EC alloantibodies in settings of MHC-mismatched and MHC-matched (non-MHC-mismatched) allogeneic rat heart Tx. Allosera reactivity with graft EC and complement activation was detected in the graft in situ by immunohistology and *in vitro* by FACS analysis using cultures of 3 well characterized rat-heart-endothelial-cell (RHEC) lines with the appropriate donor MHC and non-MHC haplotype [14]. Our results show that in MHC-mismatched and also in MHC-matched heart Tx, alloantibodies reactive with graft EC are generated. In situ and in vitro studies showed that the various IgG subclasses, among them the complement-fixing ones, were represented in the alloantibody response against MHC and also against non-MHC alloantigens on graft EC. Also, in clinical studies allosera reactivity with endothelial alloantigens in heart allograft rejection has been described. However, in these studies discrimination between HLA and non-HLA alloantigens was not further elucidated [19, 20]. Both the anti MHC and anti non-MHC allosera showed reactivity with normal, non-stimulated, heart EC cultures but also with cytokine-stimulated EC. Anti MHC allosera reactivity was increased with cytokine-stimulated EC, whereas for anti non-MHC allosera no differences in reactivity with non-stimulated and cytokine-stimulated EC were found. The fact that in situ alloantibody deposition was observed along the entire graft vasculature and that in vitro no differences in allosera reactivity with the 3 RHEC lines was observed, indicates the uniformity of MHC and non-MHC alloantigen expression in the vascular EC population of the graft.

Our FACS analysis studies on C3 and C5b-9 formation by allosera on EC cultures showed distinct C3 formation by anti MHC allosera. With anti non-MHC allosera minor C3 expression was noticed only with GP complement. Furthermore, in previous studies on alloantibody's potential to activate complement in renal allograft rejection, superiority of GP complement over rat

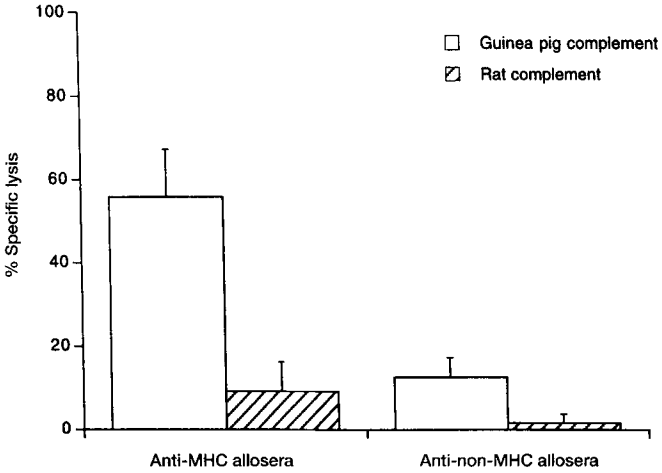

Fig. 3 Anti non-MHC allosera not very cytotoxic for EC. ^{51} Cr cytotoxicity tests with allosera were executed with TNFα + IFNγ (24–48 h) -stimulated EC from RHEC 3 in the presence of GP complement (*open bars*). The *striped bars* show the much lower cytotoxicity of the allosera when incubated with rat in stead of GP complement. Experiments were executed with a series of 2 anti non-MHC and 2 anti MHC allosera. Values are means with standard deviation of 3 experiments. Specific lysis by the allosera is given as a percentage of the lysis obtained with hyper immune serum (collected from BN rats immunized with Lewis lymph node cells) and GP complement

complement was observed [4]. This lower complement activation could have been effected by endothelial complement control proteins (CCP) such as decay accelerating factor and CD 59, which are known to prevent complement activation on homologous but not heterologous cell membranes [12, 22]. However, our studies, in which GP- and rat complement were both tested in a xenogeneic system, showed the superiotity of GP- over rat complement and suggest that the lower cytotoxicity effects measured with rat complement are not likely due to inhibition of complement activation by syngeneic CCP. That the relatively high complement activation obtained with GP serum may be effected by the presence of natural (xeno)antibodies to rat in the GP serum, is also unlikely, since the GP serum was agarose-adsorbed. C5b-9 formation, which could technically only be measured in the rat complement system, was low, and no differences between immune serum, anti MHC, and anti non-MHC, serum were found. Since these sera showed differences in complement-mediated lytic potentials, it suggests that C5b-9 expression as measured by FACS analysis is not representative for lytic MAC formation.

In situ, C3 expression could be observed in the vasculature of the rejected MHC-mismatched hearts, but not in that of the MHC-matched hearts. These data on C3 expression in situ are consistent with the in vitro results on rat C3 activation as obtained with the allosera. Although C3 expression in MHC-mismatched allografts was obvious, no C5b-9 could be detected. Since in vitro anti-MHC allosera with rat complement did effect some EC lysis, some C5b-9 expression in the allograft was expected. It may be that the immunoperoxidase staining applied to detect C5b-9 is not sensitive enough in this respect. In our study, complement-mediated cytotoxicity did correlate with C3 expression. Apparently distinct C3 expression on EC is representative for eventual formation of lytic C5b-9. Also in clinical heart or kidney Tx studies, classical complement activation in the vasculature and complement-mediated cytotoxicity by anti-EC antibodies has been described [21, 27, 29]. Other studies show that C5b-9 formation may effect, in addition to cell lysis, other processes such as production of platelet activating factor, tissue factor, and cytokines relevant for development of vasculopathy [2, 5, 6, 7, 28, 32]. In this respect, the difference in complement activation potentials between anti MHC and anti non-MHC allosera is striking, since anti non-MHC allosera do contain allo IgG antibodies of the various IgG subclasses (including the best complement-fixing IgG2b subclass [34]. The IgG subclass profiles of anti MHC and anti non-MHC alloantibodies were rather similar (data not shown), which suggests that low complement activation by anti non-MHC allosera is not likely to be due to low titers of complement-fixing IgG subclasses, but presumably to low antigen density of non-MHC alloantigens. In renal allograft rejection, furthermore, alloantibodies reactive with non-MHC alloantigens on graft EC have been described [18, 23, 24, 37], but defined information on complement activation is lacking in these studies.

In clinical heart- and kidney transplantation studies, the presence of anti EC alloantibodies has been associated with chronic rejection and, in particular, vascular rejection, including graft arteriosclerosis [17, 24, 37]. It is interesting that in our MHC-matched heart Tx model, in which grafts are rejected with delay, the development of arteriosclerotic-like lesions is associated with an alloantibody profile consisting of anti EC IgG alloantibodies with low complement activating and lytic effects. These antibodies may mediate other than complement-mediated processes relevant for development of vascular rejection including transplantation-associated arteriosclerosis. In this respect one may specifically suspect interactions with Fc-receptor positive inflammatory cells leading to processes such as antibody-dependent cell cytotoxicity (ADCC), leukocyte adhesion and infiltration with production of relevant cytokines and other inflammatory mediators.

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