A CD31-Derived Peptide Prevents the Development of Antibody-Mediated Lesions in a Rat Model of Aortic Allograft

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ABSTRACT

Background. Antibody-mediated rejection (AMR) is a major cause of graft loss. The development of donor-specific antibodies (DSAs) directed against the allogeneic HLA molecules expressed by the graft also leads to accelerated arteriosclerosis. CD31 is a protein expressed on endothelial and immune cells, ensuring homeostasis at this interface. When strong immune stimulation occurs, the regulatory function of CD31 is lost owing to cleavage of its extracellular portion. P8RI, a synthetic peptide that binds to the ectodomain of CD31, is able to restore the CD31 immunomodulatory function. In this study, we hypothesized that CD31 could represent an attractive molecular target in AMR and investigated whether P8RI could prevent the development of vascular antibody-mediated lesions.

Materials and methods. A rat model of orthotopic aortic allograft was used, and P8RI was administered for 28 days. Circulating DSAs were quantified to assess the alloimmune humoral response, and histologic and immunohistochemical analyses of aortic allografts were performed to estimate antibody-mediated lesions in the allograft.

Results. Aorta-allografted rats receiving P8RI developed fewer DSAs than control animals (mean fluorescence intensity 344 vs 741). The density of nuclei in the media (3.4×10^{-5} vs 2.2×10^{-5} nuclei/px^2) and media surface area (2.33×10^6 vs 2.02×10^6 px^2) were higher in animals treated with P8RI than in control animals.

Conclusions. These data support a therapeutic potential for molecules able to restore the CD31 signaling to fight AMR. P8RI, an agonist synthetic peptide targeting CD31, might prevent DSA production and have a beneficial effect in limiting arterial antibody-mediated lesions. CD31 agonists may become therapeutic tools to prevent and treat solid organ transplant arteriosclerosis.

ACCELERATED arteriosclerosis of solid organ transplants is mediated by the development of donor-specific antibodies (DSAs) directed against the allogeneic HLA molecules expressed by the graft. Immunosuppressive drugs are insufficient to prevent the generation of DSAs. As a result, antibody-mediated rejection (AMR) has emerged as a major cause of graft loss.

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The development of antigen-specific antibodies requires the activation of antigen-presenting cells as well as of T helper and B lymphocytes, the coordination of which is physiologically controlled by cell-surface immunoregulatory molecules. Among the latter, CD31 is particularly interesting as a potential therapeutic target for AMR because it is expressed by all the immune cells and its regulatory functions are engaged by transhomophilic interaction. CD31, also known as platelet endothelial cell adhesion molecule-1, is a transmembrane Ig-like immunoreceptor tyrosine-based inhibition motif protein expressed on endothelial cells, platelets, and leukocytes. This inhibitory signaling receptor ensures homeostasis at the interface between immune cells and blood vessel cells. When strong immune stimulation occurs, the regulatory function of CD31 is lost owing to cell activation–induced cleavage of its extracellular portion [1]. However, as CD31 is partially but not completely lost on activated cells, its incomplete cleavage provides the opportunity to rescue its immunomodulatory function by targeting the residual portion of the molecule. In this setting, P8RI, an agonist synthetic peptide that binds to the juxtamembrane amino acid sequence of the ectodomain of CD31, shows an immunosuppressive effect through restoration of the CD31 inhibitory pathway [1–3].

In this study, we hypothesized that CD31 could represent an attractive molecular target in AMR and investigated whether P8RI could prevent the development of vascular antibody-mediated lesions in a murine model of orthotopic aortic allograft (AA).

MATERIALS AND METHODS

Seven-week-old Lewis (LEW; RT1l) rats were used as recipients, and age-matched male Brown-Norway (BN; RT1n) rats were used as allogeneic donors in a model of AA, as described in Mennander and Häyry [4] and Thaunat et al [5]. All experiments conformed to Directive 2010/63/EU of the European Parliament, and approval was obtained from the Paris Nord Animal Ethics Committee. A 1 cm long segment of the donor BN abdominal aorta was transplanted in the orthotopic position by end-to-end anastomosis in the recipient subrenal aorta. Rats were induced with 4% isoflurane and then maintained on 2% isoflurane. Intraperitoneal injection of buprenorphine was used for analgesia (0.03 mg/kg preoperatively and postoperatively every 8 hours). P8RI was administered subcutaneously for 28 days at 2.5 mg/kg/d, and control animals received subcutaneous injections of phosphate buffered saline.

Blood was collected in dry tubes to retrieve serum 28 days after AA. The animals were sacrificed by intraperitoneal injection of sodium pentobarbital. Aortas were snap frozen in liquid nitrogen. Longitudinal cryosections were cut at 5 μm thickness in optimal cutting temperature medium, stained with 4′,6-diamidino-2-phenylindole and orcein. Density of nuclei in the media and media surface area were measured in 10 fields per aorta at x20 magnification and were quantified using a computerized morphometric analysis system (Qwin Software, Leica Microsystems, Nanterre, France). Immunofluorescence staining of AAs was performed using the following antibodies: polyclonal alpha-smooth muscle actin (alpha-SMA) and IgG, monoclonal CD68 (clone ED-1). Apoptosis was identified by the terminal deoxynucleotidyl transferase (TdT) dUTP nick end labeling assay. Detection of circulating DSAs was performed in LEW recipients 28 days after AA by adding LEW sera on fresh BN splenocytes mixed with preimmune LEW sera. After incubation with IgG-AF647 and CD45R-FITC, IgG MFI was measured on CD45R-negative cells, using a LSRII flow cytometer and BD FACSDiva software 6.0 (BD Biosciences, San Jose, Calif, United States).

Statistical analyses were performed using GraphPad Prism 6.0 (GraphPad Software, San Diego, Calif). The differences between groups were evaluated using Mann-Whitney *U* test and analysis of variance, as appropriate. The differences were considered significant when the P value was $< .05$.

RESULTS

Antibody-mediated medial remodeling is observed in allogeneic AA model

Disappearance of alpha-SMA+ smooth muscle cells (SMCs) was observed in the AAs and was associated with marked apoptosis in the media and ED-1+ macrophage infiltration (Fig 1A). IgG binding to SMCs was observed in the media and colocalized with apoptotic TUNEL+ cells (Fig 1B). These data validate the allogeneic AA as an accurate model of AMR, in which antibodies binding to donor SMCs are responsible for medial apoptosis and remodeling, as previously described [4,5].

P8RI reduces DSA titers in an allogeneic AA model

Aorta-allografted rats receiving P8RI for 28 days from allograft developed fewer DSAs than aorta-allografted control animals (MFI 344 vs 741, $P = .03$; Fig 1C).

P8RI prevents the development of DSA-mediated lesions in the allograft

Analysis of aortic grafts showed that density of nuclei in the media (3.4×10^{-5} vs 2.2×10^{-5} nuclei/px^2, $P = .01$; Fig 1D) and media surface area (2.33×10^6 vs 2.02×10^6 px^2, $P = .007$; Fig 1E) were higher in animals treated with P8RI than in control animals. Intimal surface area was not different between P8RI and control animals (0.53×10^6 vs 0.62×10^6 px^2).

CONCLUSIONS

CD31 is a protein expressed on endothelial cells, platelets, and leukocytes, ensuring homeostasis at the interface between immune cells and blood vessel cells. As CD31 is partially, but not completely, lost on activated cells on immune stimulation, this cleavage provides the opportunity to rescue its immunomodulatory function by targeting the residual portion of the molecule. In this setting, the agonist synthetic peptide P8RI shows an immunosuppressive effect in inflammatory processes, such as those underlying progression of atherosclerosis [3]. In solid organ transplants,

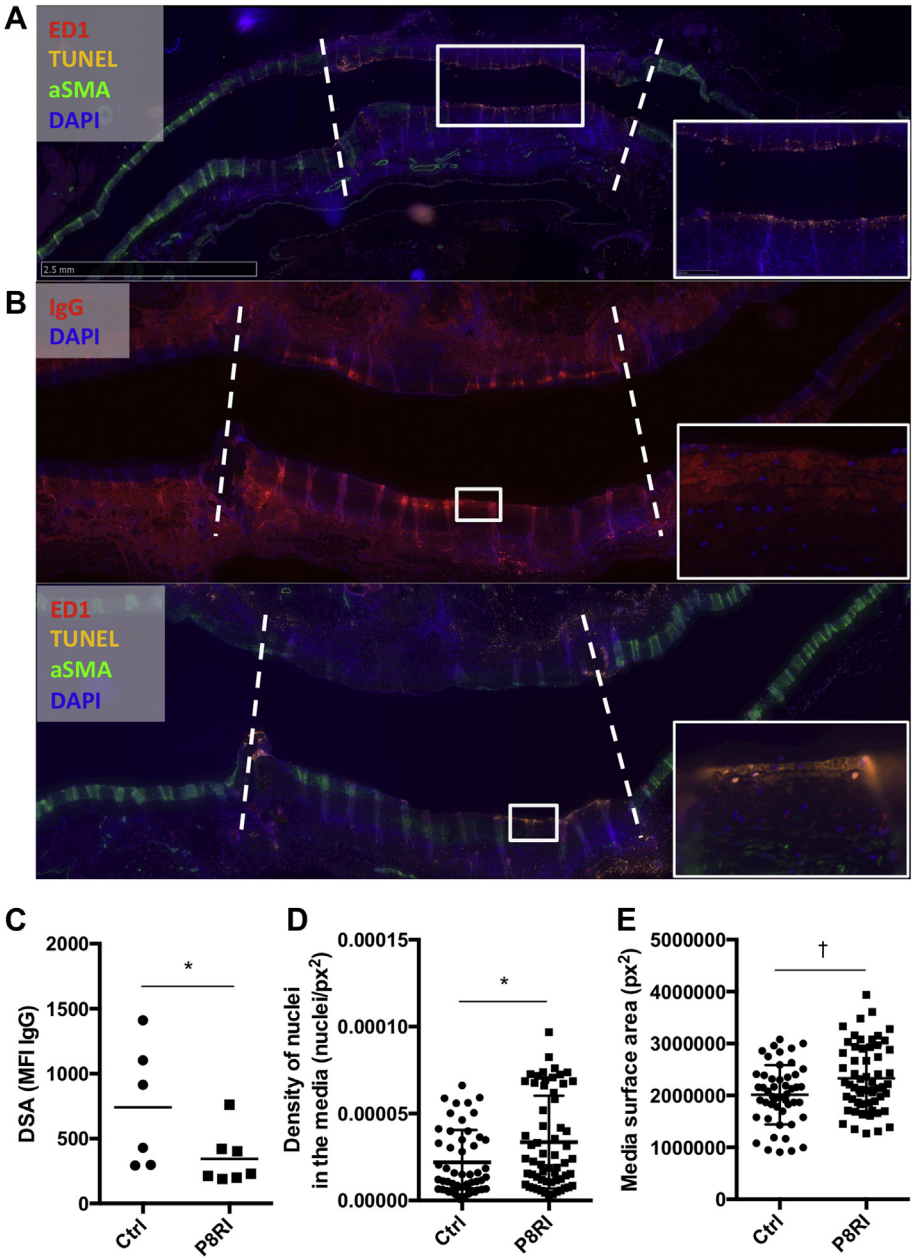

Fig 1. P8RI prevents vascular antibody-mediated lesions observed in aortic allograft. (A) Disappearance of alpha-smooth muscle actin (alpha-SMA) + smooth muscle cells (SMCs) was observed in the aortic allograft and associated with marked apoptosis in the media and ED-1+ macrophage infiltration. (B) IgG binding to SMCs was observed in the media, many of SMCs being 4′,6-diamidino-2-phenylindole negative and colocalized with apoptotic TUNEL+ cells. (C) Quantification of donor-specific antibodies estimated by the IgG MFI in control (n = 6) and P8RI groups (n = 7) ($P < .05$ by Mann-Whitney *U* test). (D) Density of nuclei in the media, measured in 10 fields at x20 magnification per animal, was higher in P8RI than in the control group ($P < .05$ by analysis of variance). (E) Media surface area, measured in 10 fields at x20 magnification per animal, was higher in P8RI than in the control group ($P < .01$ by analysis of variance). *$P < .05$, †$P < .01$.

the development of DSAs and the occurrence of AMR directly contribute to accelerated arteriosclerosis [5]. We herein provide experimental data supporting a therapeutic potential for molecules able to restore the CD31 signaling to fight AMR and transplant arteriosclerosis. P8RI, an agonist synthetic peptide targeting CD31, might not only

prevent DSA production but also have a beneficial effect on limiting arterial antibody-mediated lesions, as suggested by our findings in a murine model of AA. CD31 agonists may become important therapeutic tools to prevent and treat solid organ transplant arteriosclerosis.

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