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Brief communication

A pig allograft model of antibody-mediated rejection

Paul E. Herbert [a,c], M. Finn Morgan [a,c], Ivan Berton [a], Robert I. Lechler [a], Anthony Dorling [a], Gordon Williams [c], Anthony N. Warrens [a,b,*]

[a] Department of Immunology, Imperial College London, Hammersmith Campus, Ducane Road, London W12 0HS, UK
[b] Department of Renal Medicine, Imperial College London, Hammersmith Campus, Ducane Road, London W12 0HS, UK
[c] Department of Surgery, Hammersmith Hospital, Ducane Road, London W12 0NN, UK

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ABSTRACT

Allograft rejection caused by antibodies in sensitised individuals remains a real problem in human allotransplantation. There would be value in a simple model of this process to evaluate the mechanisms involved in antibody-mediated damage and the development of accommodation, as well as the impact of potential interventions. We have thus developed a novel large animal model of this process using an allosensitisation system. Two inbred lines of miniature pigs that carry different major histocompatibility antigen haplotypes were used. Pigs of one line were sensitised by the sequential subcutaneous injection of major histocompatibility antigen-mismatched allogeneic peripheral blood mononuclear cells derived from the other inbred line. We demonstrated that this generated high titres of allospecific antibodies. We then transplanted carotid arteries from donors syngeneic to the priming cells into the sensitised animals. After 48 h these vessels showed a profound mononuclear cell inflammatory infiltrate in both intima and media, fibrin deposition, and luminal compromise with thrombus and antibody deposition. The mean endothelial surface affected by this process was 59.2%. No such pathology was seen in any of the controls. This model is technically simple to perform with few post-operative complications. It provides proof-of-principle of a model of antibody-mediated rejection which will be of potential value in elucidating the mechanisms underlying the process and the efficacy of interventions to prevent or treat it.

1. Introduction

Pre-formed antibodies are a significant issue in allotransplantation, when the transplantation of sensitised recipients with pre-formed HLA-specific antibodies is being considered. In such situations, attempts to remove antibodies in advance of and following transplantation, combined with strategies to suppress further production of anti-HLA antibodies have met with some success [1–3]. However, the outcome in these situations is still inferior to transplantation in the non-sensitised recipient and the parameters that determine success have not been well defined. The situation is further complicated by the observation that, in some situations, the return of antibodies following transplantation does not cause the expected damage, because the graft has in some way adapted itself to be less susceptible to antibody-mediated damage, a process called "accommodation" [4,5]. In addition, new, highly sensitive techniques are now available that can identify the presence of low, previously undetected levels of antibodies [6,7]. Hence, there are several variables that need to be teased out to understand the mechanisms underlying these phenomena. It is likely that they will only be elucidated using an appropriate animal model. Rodent models of vascular transplantation have been described, sometimes demonstrating the role of antibodies in transplant vasculopathy, but are technically difficult [8–10]. That is why we have developed a large animal model.

2. Objective

In this study, we sought to prove the principle that it would be possible to study antibody-mediated allograft damage using a technically easy, large artery model in the pig. To do this we have studied a system with high levels of pre-formed allospecific antibodies. Having proved this principle, we believe this model will be useful to study the importance of different levels of antibodies, different timing of induction (before and after transplantation) and the mechanisms underlying different outcomes (rejection and accommodation).

3. Materials and methods

3.1. Animals

Inbred lines of minipigs (c/c and d/d SLA (swine leucocyte antigen) phenotypes) [11] (kind gift of Dr David Sachs) were used. c/c

* Corresponding author. Department of Immunology, Imperial College London, Hammersmith Campus, Du Cane Road, London, W12 0HS, UK. Tel.: +44 20 8383 2307; fax: +44 20 8383 2788.
E-mail address: a.warrens@imperial.ac.uk (A.N. Warrens).

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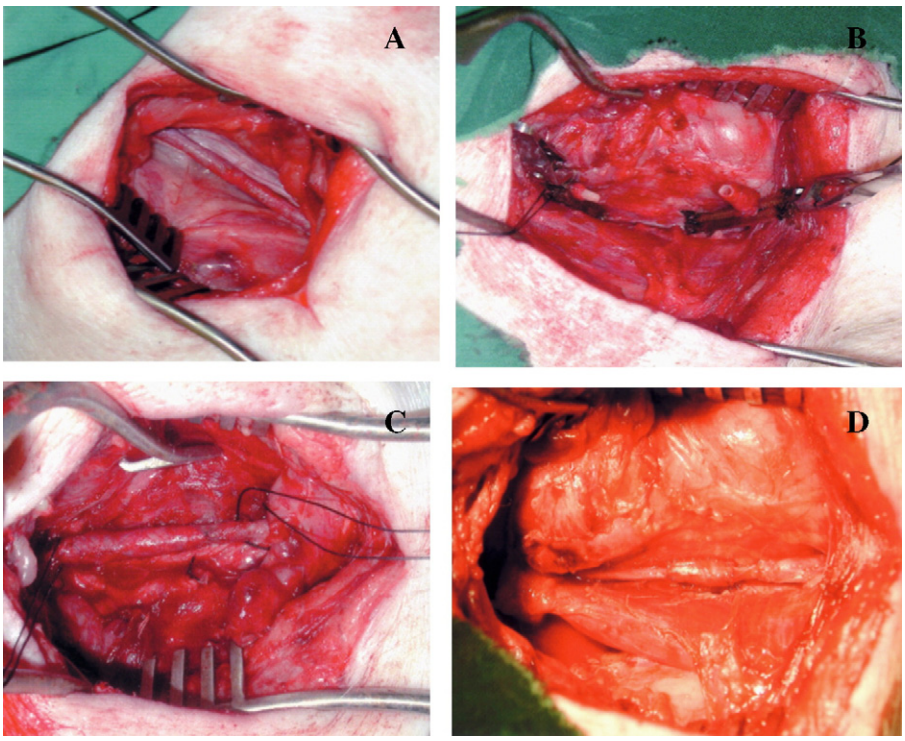

Fig. 1. Porcine carotid artery transplantation. The carotid artery was exposed (A) and a section excised (B). Another section of artery was transplanted with two end-to-end anastomoses (C) and the animal was allowed to wake up. Forty-eight hours later the vessel was exposed (D) and excised. Further details of the surgical methods involved are provided in Materials and methods.

animals were used as recipients of vessels (or untransplanted controls) and both c/c and d/d animals as vessel donors. All were greater than 20 kg at use. All procedures were performed in accordance with the Animals (Scientific Procedures) Act 1986, after satisfying the internal ethical review process of Imperial College London.

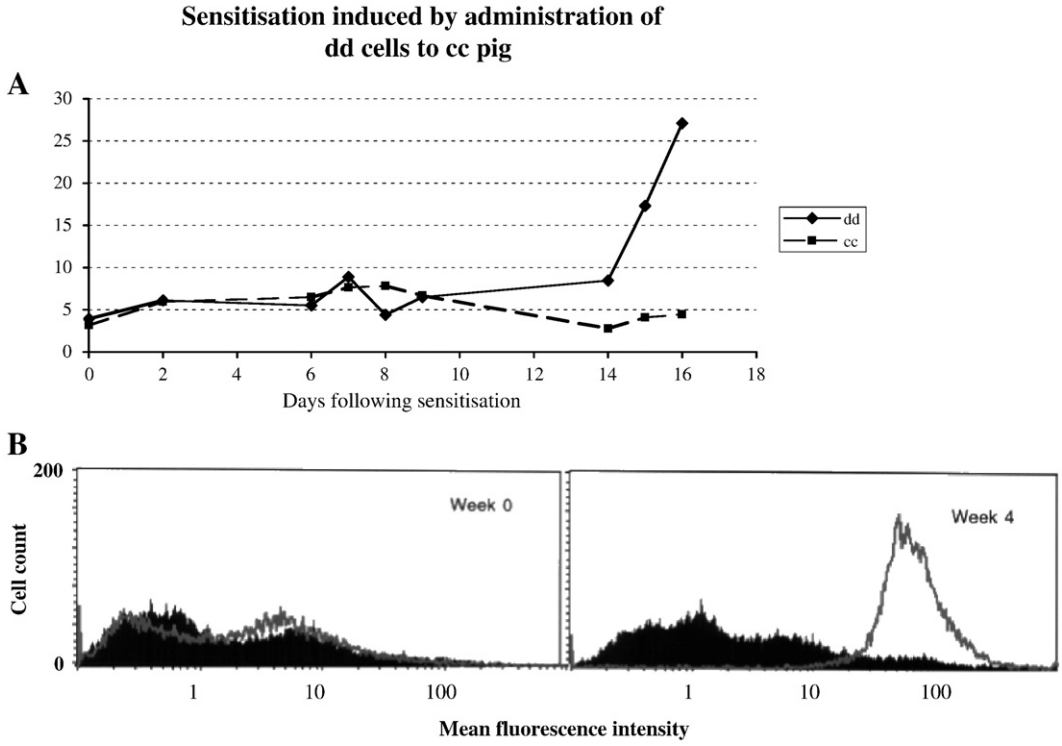

Fig. 2. Generation of SLA^d-allospecific antibodies in SLA^c pigs. SLA^c pigs were sensitised with weekly injections of SLA^d cells (days 0, 7, 14 and 21) or not (controls). On regular occasions, blood was drawn and neat serum mixed with either SLA^d (dd) (solid line) or control SLA^c (cc) (broken line) cells and FITC-conjugated goat anti-pig IgG added. (A) Allospecific antibody is detectable by day 16. (B) High titres are observed after four weeks. In these flow cytograms, signal from SLA^d cells are shown in outline and from control SLA^c cells in solid graphs. This represents a representative example of four pigs.