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Immunomodulation of dog islets using a cocktail of monoclonal antibodies

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Abstract. Islet allografts are particularly vulnerable to rejection, and current immunosuppressive agents are deleterious to their function. They are, however, highly suitable for 'immunomodulation', i.e., the removal or inactivation of passenger leukocytes to reduce their immunogenicity. For this purpose we have used 3 rat anti-dog monoclonal antibodies (Mabs) which are synergistic for leukocytolysis in the presence of autologous dog serum. Spleen cells or purified islets treated with these Mabs together with autologous serum were tested in mixed leukocyte and islet co-culture assays. The stimulatory properties of the Mab-pretreated splenocytes or islets were markedly reduced; moreover, the Mab cytolytic activity was shown to be confined to the leukocyte target cells and did not affect islet secretory function upon glucose stimulation. We conclude that this method of modifying the immunogenicity of dog islets could lead to successful islet grafting in vivo, allowing the reduction of conventional immunosuppression. Successful in vivo studies in this model, which are currently in progress, could have implications for clinical islet transplantation.

Key words: Islet transplantation – Dog model – Immunomodulation – Monoclonal antibodies

In our Department, a postmortem specimen of a transplanted, vascularized, duct-injected segmental pancreas graft was found to consist entirely of numerous, well-granulated islets 9 years after transplantation. The patient had been insulin-independent for over 9 years and died of a myocardial infarct during dialysis. She had rejected her simultaneously transplanted kidney graft 2 years after transplantation and was subsequently haemodialysed. This example of the survival and function of islets surrounded by totally fibrosed tissue, thus resembling a vascularized islet graft, has given us confidence in pursuing islet transplantation as an alternative therapy for insulin-dependent diabetes mellitus. However, compared with vascularized pancreas transplantation, rejection of islet tissue has been shown to be more difficult to prevent with the currently available immunosuppressive therapy [4].

One approach to this problem is to reduce the immunogeneicity of the islet graft by pretreatment with monoclonal antibodies (Mabs) directed towards passenger leukocytes, in particular dendritic cells and macrophages. The host immune response should then be diminished and therefore more easily immunosuppressed. In rodents such methods are well documented and can secure long-term survival of functional islet allografts [2]. However, these procedures must be proved effective in a large animal model before serious consideration can be given to clinical application. To this end we have developed rat anti-dog Mabs with selected properties for the immunomodulation of donor islet tissue prior to transplantation into diabetic recipients. In this report we describe in vitro studies of dog islet immunomodulation using 3 Mabs which, in contrast to most mouse Mabs, have the ability to fix autologous complement and lyse target cells.

Materials and methods

Monoclonal antibodies were produced by standard techniques and characterized by FACS analysis and a battery of in vitro functional assays. From a large number of rat anti-dog hybridoma supernatants we selected 3 Mabs (one IgG2a, two IgG2b) which synergise for the cytolysis of dog peripheral leukocytes (PBL) in the presence of autologous dog serum as the complement source. One of these Mabs binds to the CD45 leukocyte common antigen (LCA); the target molecules for the other two Mabs are currently being identified.

The binding of these Mabs to dog pancreas sections was visualized by standard immunohistochemical staining techniques using peroxide or alkaline phosphatase stains.

Islet isolation. Dog islets were isolated by static, intraductal collagenase digestion and purified over Ficoll gradients according to the

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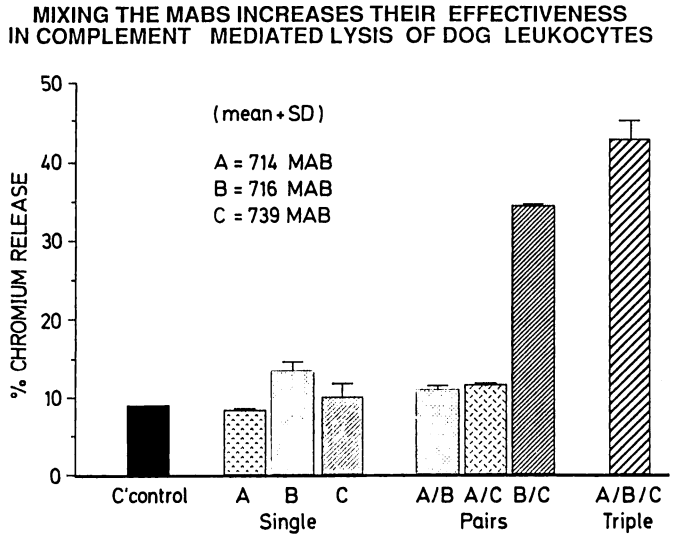

Fig. 1. Monoclonal antibodies (Mabs) were tested singly, in pairs or in triple cocktail for cytolytic activity using ^{51}Cr-labelled dog peripheral blood lymphocytes (PBL) and autologous dog serum as the source of complement. Cytolysis after 1.5 h at 37 °C was measured by chromium release expressed as percentage of total chromium in target cells

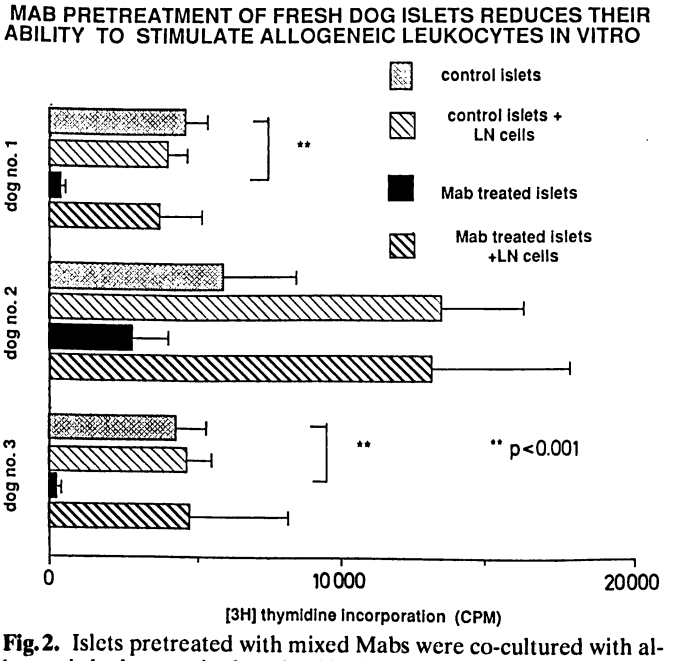

Fig. 2. Islets pretreated with mixed Mabs were co-cultured with allogeneic leukocytes in the mixed leukocyte islet co-culture (MLIC) assay. Proliferative responses were measured by titrated thymidine incorporation on day 7. Residual Mabs did not affect the responser population as seen by restored proliferation in cultures reconstituted with stimulator-type leukocytes

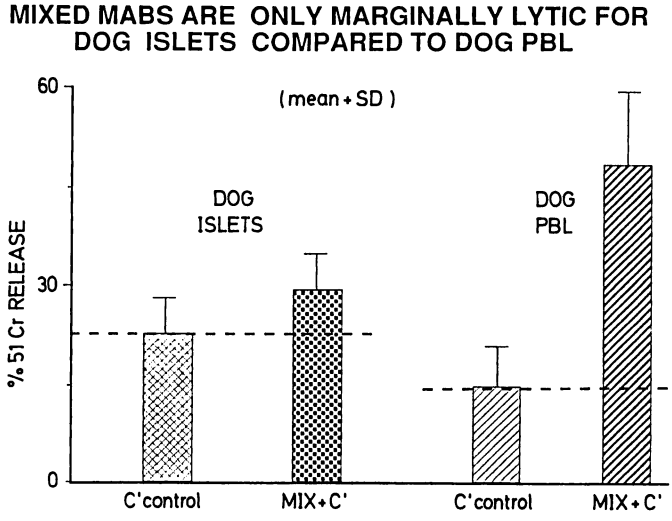

Fig. 3. Lytic activity of mixed Mabs was tested on ^{51}Cr-labelled dog islets or ^{51}Cr-labelled dog PBL with autologous dog serum as the complement source. Cytolysis after 1.5 h was measured by ^{51}Cr release as percentage of total ^{51}Cr in target cells

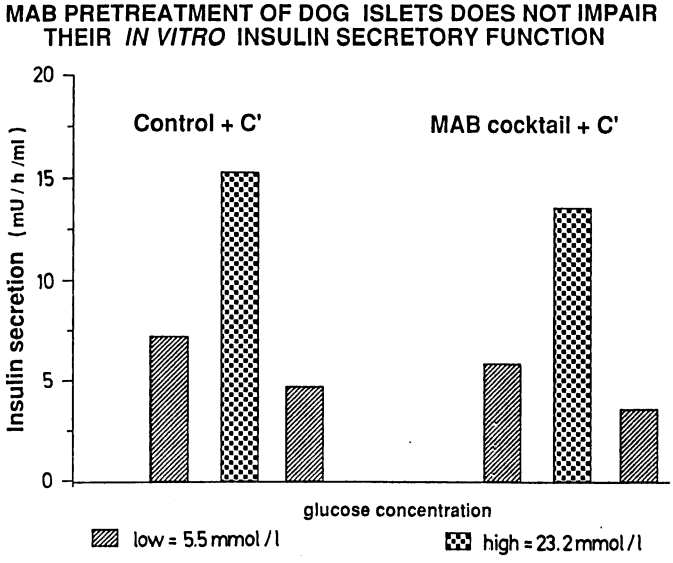

Fig. 4. Islets pretreated with mixed Mabs were incubated with low and high glucose concentrations in a static glucose stimulation assay. Insulin secretion was measured by radioimmunoassay for dog insulin

Assay methods. Chronium-51 release assays were used to measure the cytolytic activity of Mabs on ^{51}Cr-labelled dog islets or peripheral blood leukocytes (PBL). The secretory function of islets after Mab pretreatment was tested by the standard static glucose stimulation assay as compared with untreated islets.

method of Warnock et al. [5]. Dithizone stain [3] was used for islet identification and assessment of purity. The viability was assessed by acridine orange/propidium iodide staining [1]. After overnight culture, dog islets with a purity of 60%–90% were incubated with Mab(s) together with autologous dog serum as the source of complement for 18 h at 37 °C and then washed extensively. Next, 30–50 irradiated (2000 rads), pretreated islets or pretreated splenocytes (1×10^5) were added to allogeneic dog lymphocytes (1×10^5) in the mixed lymphocyte islet co-culture (MLIC) assay or the mixed lymphocyte culture (MLC) assay, respectively.

Results

The immunohistochemistry study of Mab binding to dog pancreas sections showed that none of the Mabs bound to the endocrine part of the islet. Two Mabs were found to bind to dendritic- and macrophage-type cells and passenger leukocytes distributed throughout the pancreas section. The cytolytic activity for dog PBL targets of Mabs

used singly, in pairs and as a cocktail in the ^{51}Cr-release assay is shown in Fig. 1. Whereas single Mabs showed marginal cytolysis above the complement control level, one pair of Mabs and the mixture of all 3 Mabs gave substantial target cell lysis. This triple cocktail was therefore used to pretreat splenocytes, which were then tested in MLC assays. Results showed that the stimulatory capacity of splenocytes was greatly reduced by pretreatment with the Mab cocktail. Using Mab-pretreated dog islets as stimulators in the MLIC assay, similar results were obtained, as shown in Fig. 2 (three experiments shown). There was, however, a wide range of reduced proliferative responses, possibly related to islet purity and the use of unpurified Mab in the pretreatment procedure. The effect of Mab and complement pretreatment on islet cells and their function was measured by ^{51}Cr-release and by static glucose stimulation assays. The cytolytic activity of Mabs for islet targets was only marginally above the control level compared with PBL targets, in which a substantial isotope release was measured (Fig. 3). Figure 4 shows that the pretreatment had no discernible effect on insulin secretion, which was similar to that of control untreated islets.

Discussion

Successful application to a large animal species of methods developed in rodents for islet cell immunomodulation is a vital step before clinical application can be considered. The present work describes the preliminary in vitro assessment of the effectiveness of immunomodulation with Mabs and complement in mongrel dogs and holds promise for the in vivo studies in progress.

Though the mechanisms underlying the reduced proliferation in MLC and MLIC following Mab pretreatment of stimulator splenocytes or islets are not clear, several observations can be made:

1. It is unlikely that reduced proliferation results from Mab leakage from (thoroughly washed) treated islets. Thus, reconstitution of these cultures with small numbers of stimulator-type cells restored the normal proliferative responses (Fig. 2).
2. Intact complement seems to be needed for successful immunomodulation, suggesting that lysis of leukocytes is an important mechanism, although antibody-dependent cellular cytotoxicity (ADCC) may also be involved, particularly in vivo.
3. The ability of rat Mabs to utilise autologous dog serum for cytolysis avoids the use of heterologous complement sources which could prejudice islet function through the binding of xenoantibodies.

The variability of the MLIC results could be related to the use of supernatants as a source of Mabs and to the purity of different islet cell preparations. Undoubtedly, the methodology can be improved by using titrated mixtures of these Mabs, which are now available in purified form. Other Mabs are also being tested with the aim of improving the immunomodulation.

Conclusion

The results show clearly indicate that in vitro pretreatment of fresh dog islets with a cocktail of autologous complement-fixing Mabs can result in reduced immunogenicity in MLIC assays, which are an in vitro correlate to transplant rejection. Thus, if it is possible to reduce the immunogenicity of islet grafts, then low levels of conventional immunosuppression or therapy with rat anti-dog CD4 and CD8 Mabs may well allow prolonged survival and restoration of normoglycaemia in diabetic recipients. In vivo studies using a large animal model are currently in progress.

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