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New immunosuppression with monoclonal antibody to intracellular adhesion molecule 1 (ICAM-1) in rat organ transplantation

M. Nozawa[1], I. Otsu[1], H. Kobayashi[2], T. Yamataka[2], T. Miyano[2], Y. Okumura[3], T. Tamatani[4], and M. Miyasaka[4]

[1] Department of Surgery, Meikai University, Saitama, Japan, Departments of [2] Pediatric Surgery and [3] Immunology, School of Medicine, Juntendo University, Tokyo, Japan [4] Department of Immunology, Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan

Abstract. Inbred, male Lewis rats underwent heterotopic heart allografting from F344 donor rats, or streptozocin (STZ)-induced diabetic Lewis rats underwent pancreas allografting with bladder drainage from F344 or ACI donor rats. A monoclonal antibody (MoAb) to intracellular adhesion molecule 1 (ICAM-1) was given i.p. (1.0 mg/kg) for 10 days, and its immunosuppressive potency was evaluated. The mean survival time (MST) of the heart allografts was significantly prolonged in the MoAb-treated group. Both exocrine and endocrine MST of pancreas allografts were also prolonged by MoAb administration across the minor and major histocompatibility barriers. However, complete graft tolerance was not induced. Our study demonstrated that the MoAb to ICAM-1 alone can delay the allograft rejection in rat organ transplantation.

Key words: Heart and pancreas allotransplantations – Monoclonal antibody (MoAb) – Rat – Intracellular adhesion molecule 1 (ICAM-1)

Recently, an interruption of the attachment of lymphocytes to their targets via nonspecific accessory molecules, such as lymphocyte function antigen (LFA-1) or intercellular adhesion molecule 1 (ICAM-1) gained interest as a possible method to minimize rejection responses. At present, a monoclonal antibody (MoAb) to rat ICAM-1 is available [5]. The aim of this study was to investigate the immunosupressive potency of a MoAb to ICAM-1 in in vivo experiments, i.e., rat heart and pancreas allotransplantations.

Materials and methods

Experiment 1. Male inbred Lewis rats (RT1l) underwent heterotopic heart allotransplantations (HTx) [4]. F344 rats (RT1l) were used as donors. These rats were divided into the following groups: (1) untreated HTx group ($n = 15$) and (2) ICAM-1-specific MoAb-treated HTx groups. Group 2 was further divided into 4 subgroups according to the dosage and duration of MoAb administration: (2a) 0.2 mg/kg i.p. × 10 days ($n = 2$), (2b) 0.4 mg/kg i.p. × 5 days ($n = 2$), (2c) 0.4 mg/kg i.p. × 10 days ($n = 3$), (2d) 1.0 mg/kg i.p. × 10 days ($n = 15$). The MoAb was given from the 1st postoperative day in group 2. The graft survival was observed in each group.

Experiment 2. Male Lewis rats (RT1l), which were made diabetic by streptozocin i.v. injection (60 mg/kg), underwent pancreas allotransplantation (PTx) with bladder drainage [3]. Either F344 (RT1l) or ACI (RT1a) rats were used as donors. These rats were divided into the two groups: (1) untreated PTx group and (2) ICAM-1-specific MoAb-treated PTx group (1.0 mg/kg i.p. × 10 days). The ICAM-1-specific MoAb was given from the day of PTx. Each PTx rat was fed ad lib. in a metabolic cage. The blood sugar (BS) level and 24-h urinary amylase excretion were monitored daily. Furthermore, the groups whose grafts were harvested from ACI rats were subjected to biopsies of the grafted pancreas at day 5 after PTx for microscopic examinations (H&E staining).

In both experiments, statistical evaluations were done by Student's *t*-test or Cochran-Cox test, and the values were expressed as mean ± SE.

Results

In Experiment 1, the MST of the heart graft in the untreated group was 10 ± 1.5 days. The graft survival was not prolonged in subgroups 2a and 2b. When the MoAb was given at a dosage of 0.4 mg/kg for 10 days (group 2c), the

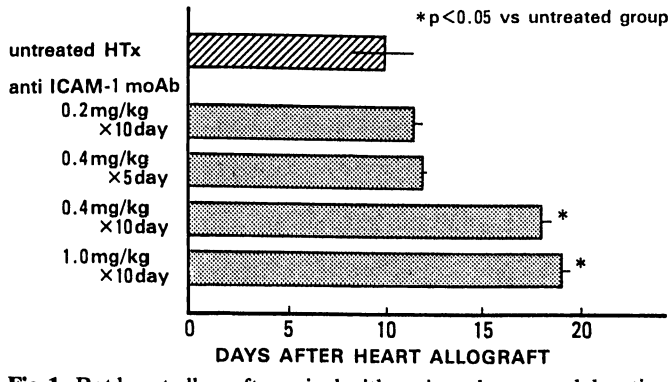

Fig. 1. Rat heart allograft survival with various dosage and duration of rat intercellular adhesion molecule 1 (ICAM-1)-specific monoclonal antibody (MoAb) administration (F344 to Lewis)

Offprint requests to: M. Nozawa, M. D., Department of Surgery, Meikai University, 1-1 Keyakidai, Sakado, Saitama 350-02, Japan

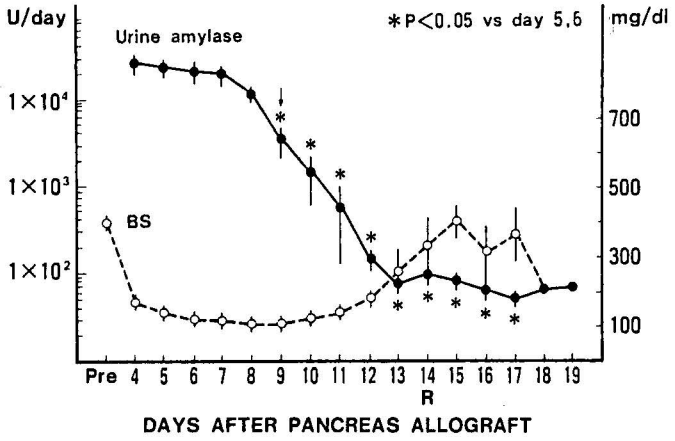

Fig. 2. The 24-h urine amylase and blood sugar *(BS)* changes in untreated pancreas allografted (PTx) rats (F344 to diabetic Lewis, $n = 7$). *Arrow*, rejection of exocrine function of graft pancreas; *R*, rejection of endocrine function

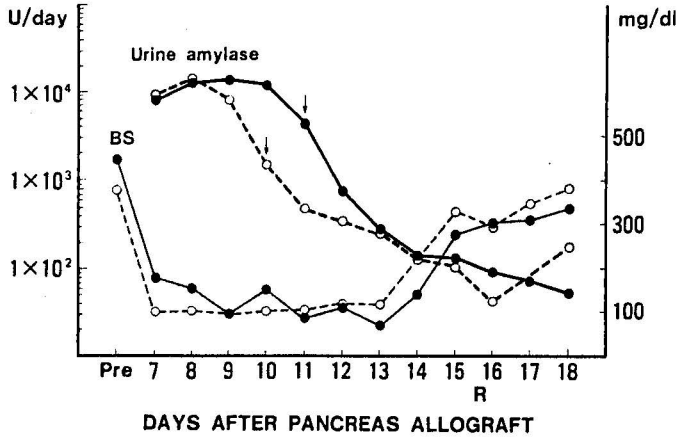

Fig. 3. The 24-h urine amylase and BS changes in PTx rats (F344 to diabetic Lewis) with ICAM-1-specific MoAb treatment (1.0 mg/kg for 10 days)

MST was 18.5 ± 0.5 days, which was significantly longer than the untreated group. When the MoAb was given at 1.0 mg/kg for 10 days (group 2d), the MST reached 19 ± 0.4 days (Fig. 1). The histology examination on day 5 after HTx revealed that the cell infiltration was less prominent, and the myocardial structures of the grafted heart were preserved in group 2d when compared with the untreated group.

In Experiment 2, in the F344 and Lewis combination, the 24-h urine amylase excretion decreased on day 9 after PTx, and the BS level increased to over 300 mg/dl on day 14 in the untreated group (Fig. 2). However, a drop in urine amylase excretion occurred either on day 10 or 11, and hyperglycemia appeared on day 15 or 16 in the MoAb-treated group (Fig. 3). When rejection of the exocrine function (a significant drop in urine amylase excretion) and endocrine function (hyperglycemia over 300 mg/dl) of the pancreas graft was observed in each PTx rat, the MST of these functions in the untreated group ($n = 7$) were 8.4 ± 0.3 days and 14.0 ± 0.6 days, respectively, whereas in the MoAb-treated group ($n = 2$), they were 10.5 ± 0.5 days and 15.5 ± 0.5 days, respectively.

In the combination of ACI to Lewis rats, the exocrine and endocrine MST of the pancreas graft in the untreated group ($n = 5$) were 6.2 ± 0.2 days and 7.5 ± 0.3 days, whereas those in the MoAb-treated group ($n = 5$) were 7.2 ± 0.4 days and 9.6 ± 0.2 days, respectively; the values were significantly longer than in the untreated group ($P < 0.05$). According to the microscopy examination of the graft on day 5, cellular infiltration was suppressed in the MoAb-treated group (Fig. 4).

Discussion

ICAM-1 is a 90-kD glycoprotein, expressed on the vascular endothelium, lymphocytes, macrophages and many other cells. The interaction of LFA-1 and ICAM-1 is re-

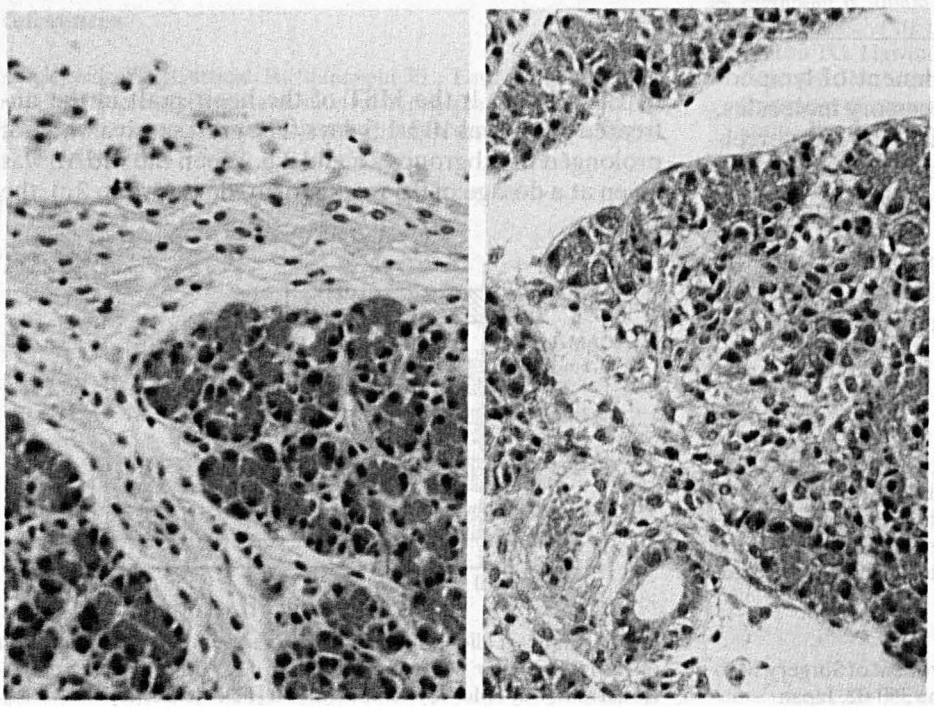

Fig. 4. Microscopy findings of pancreas allograft (ACI to diabetic Lewis rat) on day 5 after PTx (H&E, × 100). *Left*, untreated PTx rat; *right*, MoAb-treated PTx rat

quired for optimal T-cell function, which may play an important role in rejection responses. The MoAb to ICAM-1 has been used in large animal experiments [1, 2], but its immunosuppressive potency as a single agent in small animals has not been investigated. In our experiment, we administered rat ICAM-1-specific MoAb to the rats which underwent heterotopic heart or pancreas allografting and evaluated its immunosuppressive effects.

In rat heart allografts from F344 to Lewis, i.p. injection of more than 0.4 mg/kg of ICAM-1-specific MoAb for 10 days was necessary to achieve a significant prolongation of graft survival. When the MoAb was given at 1.0 mg/kg for 10 days, the MST was maximal.

Taking the result of the HTx into account, 1.0 mg/kg of ICAM-1-specific MoAb was given for 10 days after a rat pancreas allografting in order to obtain the maximum effect. In the F344 to Lewis combination, both exocrine and endocrine MST of the pancreas graft were longer in the MoAb-treated group than in the untreated group. We also examined the effect of the ICAM-1-specific MoAb across the major histocompatibility barriers. In the ACI to Lewis combination, both exocrine and endocrine MST of the pancreas graft were prolonged significantly in the MoAb-treated group when compared with those in the untreated group. Histological examination confirmed a suppression of cellular infiltration of the graft in the MoAb-treated group on the 5th postoperative day.

These results demonstrate that the ICAM-1-specific MoAb alone could prolong the graft survival in rat organ transplantations, probably through suppressing the initial rejection responses, across both minor and major histocompatibility barriers.

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