Dynamic Expression of Qa-2 during Acute Graft Rejection

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Human leukocyte antigen (HLA)-G exhibits immunotolerogenicity and is related to allograft acceptance. Qa-2 is the murine homolog of HLA-G; it has structure and functions similar to those of HLA-G. We investigated the dynamic expression of Qa-2 in skin allografts by immunohistochemistry and on peripheral blood lymphocyte subsets by flow cytometry during the entire process of acute graft rejection (AGR) with a murine skin transplantation model to determine its relationship with the pathological changes of allografts and the influence of immunosuppressive therapy. In grafts without immunosuppressive treatment, Qa-2 did not exhibit obvious changes in syngeneic and allogeneic recipients. In contrast, with immunosuppressant-treated grafts, positive expression of Qa-2 was observed. It remained at high levels in the immunosuppressant-treated syngeneic group; however, it became weakly positive and even negative in infiltrating inflammatory cells as AGR advanced, but it remained strongly positive in other skin tissues throughout the AGR process. Qa-2 expression on CD4$^+$ and CD8$^+$ peripheral blood lymphocyte subsets remained stable at a normal level in the non–immunosuppressant-treated syngeneic group. Immunosuppressive treatment can also significantly upregulate Qa-2. In the allogeneic groups, decreased expression was observed when AGR was at histological grades 1 to 2 (well before gross rejection was observed). Qa-2 was upregulated again after the graft was rejected completely. The results suggest that the increase in Qa-2 may be attributed to the use of immunosuppressive treatments. Moreover, Qa-2 expression decreased significantly with AGR progression, suggesting that it may be a potential marker for predicting AGR, especially in the presence of immunosuppressive agents.

INTRODUCTION

To date, significant improvements in clinical technologies have made solid transplantation the most effective therapeutic measure for organ failure. However, acute graft rejection (AGR) is still considered to be a major factor influencing short- and long-term allograft survival (1,2). Rational immunosuppressive therapy (3) is another known posttransplantation issue. Physicians have difficulty maintaining the balance between increasing the dosage of immunosuppressants to prevent AGR and decreasing drug levels to prevent many complications, such as viral infections and even malignant tumors.

Recently, human leukocyte antigen (HLA)-G has been introduced in transplantation studies. HLA-G is a nonclassical HLA-I molecule (4). Unlike widely expressed classical HLA molecules, HLA-G exhibits more limited polymorphism and restricted patterns of tissue expression (5). HLA-G was originally observed in fetal cytotrophoblasts during early pregnancy, where it plays an essential role in maintaining maternal-fetal immune tolerance. Further observations demonstrated its expression in other cells, including peripheral blood monocytes (6). In transplantation, the expression of HLA-G is associated with allograft acceptance. Patients with HLA-G expression in their plasma experience significantly reduced acute rejection episodes and the absence of chronic rejection compared with HLA-G–negative patients (7–9).

On the basis of the studies mentioned above, we found that many issues are of interest to this research: How does HLA-G expression on allografts or peripheral blood lymphocytes change during the process of AGR? Does immunosuppressive therapy increase the level of

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HLA-G? Is there any relationship between HLA-G expression and the pathological changes in allografts?

These questions can only be solved with animal models, because performing continuous biopsies in clinical practice is difficult. In addition, almost every recipient must be subjected to immunosuppressive treatment after organ transplantation (10,11).

The murine skin transplantation model is a broadly accepted model for the study of immunological mechanisms in allograft rejection that allows for easy surgical procedures, easy inspection, and repeatability (12). There is extensive evidence that the murine homolog of HLA-G is Qa-2 (13,14). Qa-2 in mice, as with HLA-G in humans, has been implicated in immunoregulation (15) and embryonic development (16). Qa-2$^+$ embryos have reportedly shown faster preimplantation cleavage rates, better survival and higher birth rates than Qa-2$^-$ embryos (13). To the best of our knowledge, Qa-2 expression during AGR has not been investigated.

This study aimed to follow the changes in Qa-2 expression in allografts and on different subsets of peripheral blood lymphocyte subsets (PBLs) with or without immunosuppressive treatment during the entire process of AGR to investigate the various effects of allograft rejection and immunosuppressive treatment.

MATERIALS AND METHODS

Animals

Male mice of the inbred strains C57BL/6J (H-2^b) and BALB/c (H-2^d), 8–12 wks old and weighing 23 ± 2 g, were purchased from the Institute of Laboratory Animal Science, Chinese Academy of Medical Sciences and Peking Union Medical College. The mice were kept under specific pathogen-free conditions at the Center for New Drug Evaluation of Shandong University (Jinan, China). Each mouse was housed in an isolated plastic cage to avoid damage to bandages and grafts, with controlled light/dark cycles and food and

water *ad libitum*. All of the studies were performed in accordance with the *Principles of Laboratory Animal Care* (National Institutes of Health publication vol. 25, no. 28, revised 1996) and were approved by the Institutional Animal Care and Use Committee of Shandong University.

Skin Transplantation

C57BL/6J mice were used as recipients; BALB/c mice were used as donors in the allogeneic groups and C57BL/6J mice were used as donors in the syngeneic groups. Full-thickness skin grafts (about 1.5 × 1.5 cm^2) from the donor mice were transplanted onto the dorsal thorax of C57BL/6J recipient mice, sutured with six stitches and secured with Vaseline gauze and a bandage for 5 d.

Experimental Groups

Recipients that underwent skin transplantation were randomly divided into five groups: a control group (normal mice without any treatment, n = 5), an untreated syngeneic group (group S, n = 70), an untreated allogeneic group (group A, n = 70), an immunosuppressant-treated syngeneic group (group SI, n = 130) and an immunosuppressant-treated allogeneic group (group AI, n = 130).

Groups SI and AI were treated daily with a combined systemic immunosuppressive regime of 40 mg/kg/d of cyclosporine A (Novartis Pharma, Basel, Switzerland), 100 mg/kg/d mycophenolate mofetil (Roche Pharmaceuticals, Shanghai, China), and 30 mg/kg/d prednisolone (Shandong Xinhua Pharmaceutical Group) by gavage from 7 d before transplantation to the day the mice were sacrificed.

Mycophenolate mofetil and prednisolone were diluted with drinking water, and cyclosporine A was diluted with olive oil. Previously, we had assessed the toxicity of different doses of immunosuppressants and monitored their effects on mouse skin graft rejection to choose a safe and effective dosage of these agents.

Five mice from each group were euthanized by CO$_2$ inhalation to collect grafts

and blood samples at various time points (i.e., daily from day 2 to day 14 after transplantation in the unmodified groups and from day 2 to day 26 in the immunosuppressant-treated groups).

Macroscopic Evaluation of Graft Rejection

From day 5 onward, digital photographs were taken of each mouse to evaluate graft survival by two inspectors. Macroscopic changes in the skin grafts were also observed. More than 80% necrosis of the graft surface was considered to be complete rejection.

Histological Evaluation of Graft Rejection

Skin grafts and adjacent host skin samples were harvested and fixed in 4% paraformaldehyde. Then, the skin grafts and adjacent host skin samples were embedded in paraffin wax, cut into approximately 5-μm sections and stained with hematoxylin-eosin. The slides were reviewed in a blinded fashion by two qualified pathologists to assess the quality of the graft and rejection. These were classified into four grades (grades 0–3) according to the modified scoring system for acute mouse skin allograft rejection proposed by Cendales *et al.* (17). Grade 0 (nonspecific changes) is when little infiltration of inflammatory cells into the epidermis is observed. Grade 1 (mild rejection) is when inflammatory infiltrate is observed in the perivascular and adnexal glands, hair shaft and epidermis. Grade 2 (moderate rejection) is when, in addition to prominent inflammation, a band-like infiltration is observed just beneath the dermal-epidermal junction. Grade 3 (severe rejection) is when a significant number of infiltrating inflammatory cells are observed and keratinocytes have become necrosis, forming a space between the epidermis and the dermis. The grafts detach from the host partially or completely.
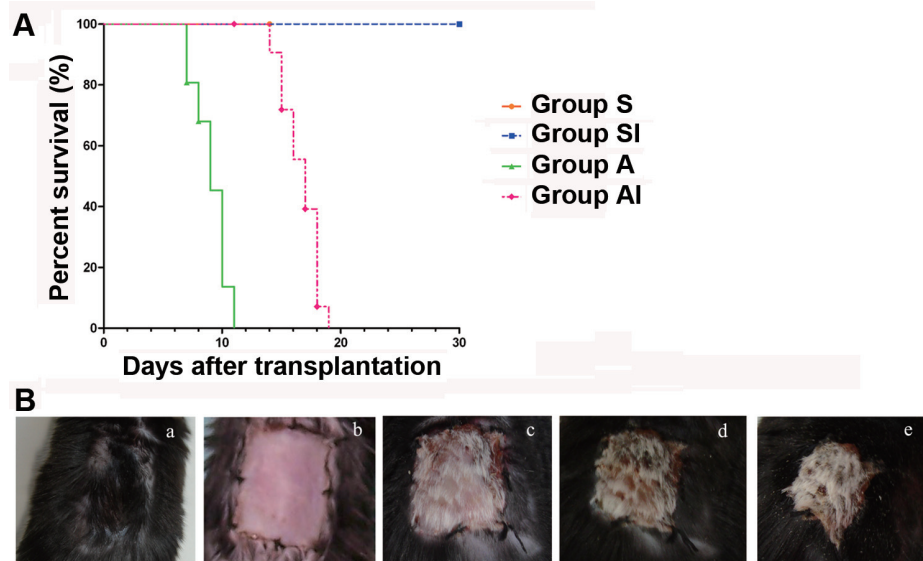
Detection of Qa-2 Expression in Grafts

The expression of Qa-2 in skin grafts was examined by immunohistochemistry

and streptavidin-peroxidase methodology with a primary antibody specific for Qa-2 (clone 69H1-9-9; eBioscience, San Diego, CA, USA) and a Histostain-Plus kit (Jingmei Biotech, Shenzhen, China) in accordance with the manual of the manufacturer. For immunostaining, the tissue sections were soaked in phosphate-buffered saline (PBS) for 5 min and in 3% H_2O_2 for 10 min to block endogenous peroxidase activity. They were then blocked with 10% normal goat serum for 30 min at room temperature and finally stained with primary antibody specific for Qa-2 (1:400) at 4°C overnight. Afterward, the sections were incubated for 30 min at 37°C with biotinylated goat antimouse IgG2a. The tissue sections were washed with PBS and incubated with avidin/streptavidin–horseradish peroxidase for 30 min at room temperature. Peroxidase activity was revealed by using a 3,3'-diaminobenzidine substrate working solution. Mouse IgG2a (eBioscience) was used for negative control staining. The sections were counterstained with hematoxylin, dehydrated in graded ethanol, cleared in xylene and finally mounted.

Flow Cytometry

Peripheral blood samples were taken with heparinized tubes when the mice were euthanized. From each sample, 100 μL was added to the polystyrene test tube (Becton Dickinson, Franklin Lakes, NJ, USA), and PE-CD4, FITC-Qa-2 and PE-Cy5-CD8 monoclonal antibodies (eBioscience) were added. FITC mouse IgG2a, PE-Rat IgG2a and PE-Cy5 Rat IgG2b (eBioscience, CA, USA) were used as isotype controls. Next, the samples were incubated for 25 min at room temperature in the dark. Red blood cells were lysed with a FACS™ lysing solution (Becton Dickinson) and then incubated for another 8–12 min at room temperature in the dark. After centrifugation for 5 min at 500g, the supernatants were discarded. Cells were washed twice in cold sheath fluid (Becton Dickinson) and then were diluted again with 100–200 μL sheath fluid. Flow cytometry was con-

ducted using a FACS Calibur instrument (Becton Dickinson). The rate of positive cells and median fluorescence intensity (MFI) of Qa-2 on peripheral blood CD4$^+$ and CD8$^+$ T-lymphocytes were calculated using CellQuest Pro software (Becton Dickinson).

Statistical Analysis

SPSS (version 13.0), GraphPad Prism (version 5.0) and Microsoft Excel (version 2003) software were used for statistical analyses. Skin graft survival results were presented as median data using Kaplan–Meier survival analysis (endpoint ≥80% graft necrosis). All numerical results were presented as means ± standard deviation (SD). Analysis of variance was used to analyze the continuous data in a group, and significant values were analyzed *post hoc* using the Student–Newman–Keuls test. In addition, a Student *t* test was used for pairwise analysis between different groups at the same time point. P values <0.05 were considered statistically significant,

and P values <0.01 were considered extremely significant.

RESULTS

Survival of Mouse Skin Grafts

All of the mice that survived throughout the experiment were healthy and had gained weight. No rejection was found in either group S or group SI for the duration of the observation period. In these groups, the grafts grew well and hair began to grow in some mice (Figure 1B, a). Grafts of the allogeneic groups became hard and black and shrank gradually until necrosis constituted >80% of the graft, which was regarded as complete gross rejection (Figure 1B, b–e). Allografts of group A were rejected within 11 d, with a mean survival time of 9 d (9 ± 0.475). The mean survival time of the skin grafts of group AI was 17 d (17 ± 0.512), much longer than that of group A (P < 0.01 versus group SI and P < 0.01 versus group A) (Figure 1A).

Figure 1. Survival of mouse skin grafts. (A) Kaplan–Meier survival curves of skin grafts from the different groups. All syngeneic grafts survived until the end of the experiment. All allografts from group A were rejected within 11 d after transplantation. The mean survival time of group AI skin grafts was much longer than that of group A skin grafts (P < 0.01 group AI versus group A). (B) Gross morphological changes of skin grafts after transplantation. (a) The syngeneic grafts grew well and were covered with hair (b–e). The allografts became hard and black and then gradually shrank and became necrotic. Complete rejection was classified as >80% of the graft surface being necrotic.

Pathological Changes of Skin Transplants

The syngeneic grafts exhibited little or no inflammatory infiltration from day 2 to day 30 after transplantation (Figure 2A).

By contrast, the allografts displayed different degrees of inflammatory infiltration and tissue destruction over time. In group A, the allografts remained at grade 0 rejection before day 4 (Figure 2B) and exhibited grade 1 inflammatory infiltration from day 5 to day 7 (Figure 2C). Between day 8 and day 11, a large number of inflammatory cells infiltrated the borderline of the grafts, indicating grade 2 rejection (Figure 2D). When allograft rejection was severe (grade 3) between day 12 and day 14 (Figure 2E), a large number of infiltrating lymphocytes and keratinocytes became necrotic, and the grafts detached from the host partially or completely to form a space between the epidermis and dermis.

In group AI, grade 1 rejection was observed from day 8 to day 12, grade 2 from day 13 to day 18 and grade 3 from day 20 until the end of the observation. Pathological AGR episodes in group AI were also markedly delayed compared with those in group A.

In the comparisons of macroscopic changes of the allografts, grades 0–1 pathological rejection correlated with almost normal-looking skin in which no macroscopic rejection was suspected. Apparent skin rejection was observed by visual examination when the pathological analysis indicated severe rejection (grade 3).

Qa-2 Expression in Skin Grafts

Skin grafts from control mice (Figure 3A) and group S (Figure 3B) usually demonstrated negative expression of Qa-2, with the exception of weak positive Qa-2 in elastic connective tissue (Figure 3A, B). Similarly, in group A, Qa-2 expression was also weakly positive in the epidermis, hair follicles and sebaceous glands during the early period of rejection classified as grades 0–1 (Figure 3C–F). As the inflammatory infiltration became more intense, the expression of Qa-2 became
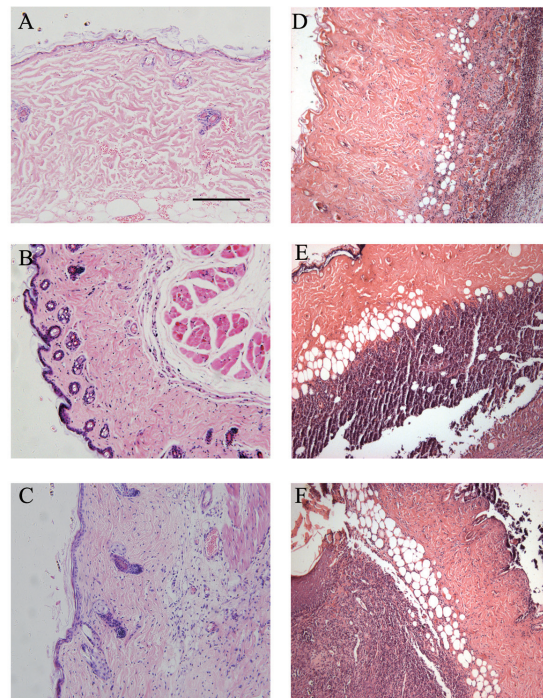

Figure 2. Pathological analysis of mouse skin grafts. Grafts were obtained from euthanized mice, fixed in 4% polyoxymethylene and then stained with hematoxylin-eosin. Specimens were classified from grade 0 to grade 3 according to their pathological changes. (A) In the syngeneic grafts, inflammatory infiltrate was almost absent at all of the time points, even on posttransplantation day 30. (B–F) In marked contrast, the allografts exhibited acute inflammation and necrosis as acute rejection progressed. (B) Grade 0: nonspecific changes. Minimal inflammatory cells are observed in the epidermis, and few lymphocytes are observed infiltrating into the superficial perivascular space and glands of the allografts. (C) Grade 1 rejection (mild rejection): diffuse infiltration of lymphocytes and monocytes is observed in the epidermis and subcutaneous tissue of the grafts. (D) Grade 2 (moderate rejection): numerous inflammatory cells infiltrate into the grafts and form infiltrating bands beneath the dermal-epidermal junction of grafts. (E, F) Grade 3 (severe rejection): a large number of infiltrating inflammatory cells is observed, and keratinocytes become necrotic and form a space between the epidermis and dermis. The grafts begin to detach from the host (E) either partially or completely, and new skin tissue replaces the interspace between the host and the graft (F). The scale bar in A is 100 μm. Original magnification: A–E, 200×.

undetectable, especially at the junction of the graft and the host (Figure 3C–F).

Effect of Immunosuppressants on the Expression of Qa-2 in the Grafts

The levels of Qa-2 expression in the skin of pretransplant recipients (Figure 3A1) and in the grafts (Figure 3B1) of group SI (treated with immunosuppressants) were significantly upregulated compared with those of the control mice and group S. Positive expression was readily detectable in the epidermis, hair follicles, sebaceous glands and dermis.

In group AI, at the beginning of rejection (grades 0–1), Qa-2 expression was strongly positive in skin tissue and infiltrating inflammatory cells (Figure 3C1, D1, E1, F1). However, as rejection advanced (grade 2), a large number of inflammatory cells exhibited negative Qa-2 expression (Figure 3E1). At the same time, other skin sites such as connective tissue retained strong positive Qa-2 expression (Figure 3E1). As allograft rejection became more severe (grade 3), the infiltrating lymphocytes retained their negative expression of Qa-2, whereas
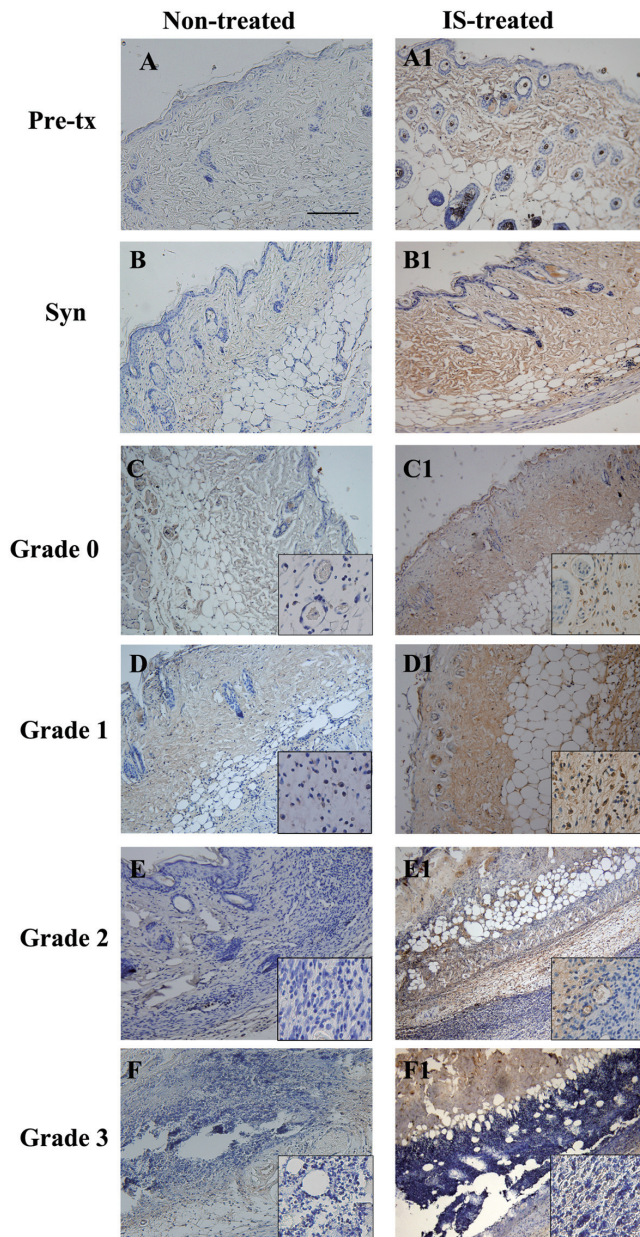
Figure 3. Qa-2 expression in the skin grafts. The expression of Qa-2 in the skin grafts was examined by immunohistochemistry with a primary antibody specific for Qa-2. Mouse IgG2a was used for negative control staining. (A, B) Grafts from pretransplantation (Pre-tx) normal mice and syngeneic mice of group S exhibited negative expression of Qa-2. (C–F) Untreated allografts exhibited weak positive Qa-2 expression in skin tissue throughout the process of rejection, and even negative expression in infiltrated inflammatory cells from grade 2 to grade 3. The panels show the staining of infiltrating cells. (A1, B1) Immunosuppressant-treated recipient and syngeneic grafts exhibited strong positive Qa-2 expression. (C1, D1) In immunosuppressant-treated allografts, Qa-2 expression also showed strong positive expression in skin tissue and infiltrating inflammatory cells during the beginning of rejection (grades 0–1). (E1, F1) When rejection was between grades 2 and 3, inflammatory cells infiltrating in the borderline of the grafts showed negative Qa-2 expression; meanwhile, in other skin sites like connective tissue, Qa-2 expression remained strongly positive. The scale bar in A is 100 μm. Original magnification: 200× for graft tissue and 400× for infiltrating cells.

other skin tissues remained strongly positive (Figure 3F1).

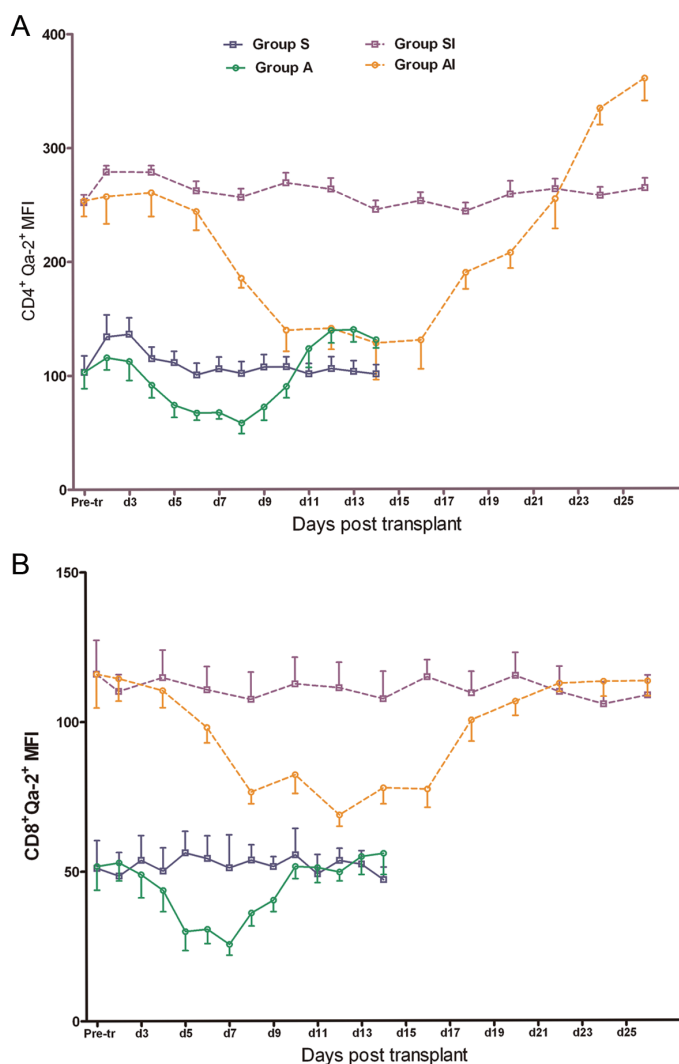## Qa-2 Expression on Peripheral CD4^{+} and CD8^{+} T Lymphocytes

In group S, Qa-2 expression remained stable during the entire posttransplantation period (that is, similar to normal levels; MFI = 103.1 ± 14.44 on CD4^{+} cells and MFI = 52.0 ± 6.97 on CD8^{+} cells, $P >$ 0.05 versus normal).

In contrast, in group A, Qa-2 expression exhibited time-dependent changes during the posttransplantation period (Figure 4). When pathological rejection was between grades 1 and 2, Qa-2 expression decreased on both CD4^{+} and CD8^{+} cells ($P < 0.01$ versus normal and group S). As rejection progressed further, Qa-2 expression was upregulated gradually. By the end of the observation period, Qa-2 expression became even higher than that in group S.

Compared to the untreated groups, Qa-2 expression in group SI and AI exhibited more obvious changes. In group SI, Qa-2 expression remained at a stable level after transplantation, but it increased significantly compared with the normal group or group S ($P < 0.01$ versus normal group and group S). In group AI, Qa-2 was at higher levels at the early stage of posttransplantation ($P < 0.01$ versus normal), similar to those of group SI ($P > 0.05$ versus group SI). However, when graft rejection was mild or moderate (grades 1 and 2), Qa-2 expression was decreased significantly in a time-dependent manner, compared with that of group SI at the same time point (see Figure 4, $P < 0.01$ versus group SI) and with the normal control level ($P > 0.05$ versus normal). From day 18 onward, Qa-2 expression gradually increased. By the end of the observation period (day 25–day 26), it was higher than the level in group SI CD4^{+} cells and as high as the level in group SI CD8^{+} cells (see Figure 4).

DISCUSSION

Currently, AGR remains the most commonly occurring complication in organ transplantation, since it induces chronic

Figure 4. Time-dependent changes in the expression of Qa-2 on PBLs during the posttransplantation period. Qa-2 expression on peripheral CD4+ and CD8+ T lymphocytes was detected by fluorescence-activated cell sorting. The dots represent means ± SD. (A) MFI of Qa-2 on CD4+ lymphocytes. In group S, Qa-2 expression remained relatively stable during the posttransplantation (except day 2–day 3) and similar to the pretransplantation level (that is, the control group level). In group A, Qa-2 expression decreased significantly from day 4 to day 9 compared with the pretransplantation level and that of group S (*P* < 0.01) before upregulation. In group SI, Qa-2 expression also remained stable but at much higher values than those in group S (*P* < 0.01). In group AI, a significant decrease in Qa-2 expression was observed from day 8 to day 20 before increasing to a level even higher than that of group SI. (B) MFI of Qa-2 on CD8+ lymphocytes. In groups S and SI, Qa-2+ expression remained relatively stable during the posttransplantation period; however, the values were much higher in group SI than in group S. In groups A and AI, when rejection was at grades 0 and 3, Qa-2 expression was similar to that during pretransplantation. A significant decrease of Qa-2 expression was observed from day 3 to day 10 and from day 5 to day 17 for groups A and AI, respectively.

graft rejection, allograft failure, and even the death of the recipient (18). Although the mechanisms of acute rejection are far from being completely understood, it has been traditionally thought that AGR is mainly a cell-mediated immune response

(19). At the onset of AGR, direct and indirect reorganization result in the proliferation and activation of naive T cells into alloreactive effector T cells. Infiltration of these alloreactive T cells and other inflammatory cells into the graft constitutes the initial stage of AGR; this occurs before any graft destruction. Therefore, the degree of inflammatory infiltration has been widely used as the criterion for classifying the histological rejection of allografts. Many researchers have devoted their studies to developing noninvasive markers that change according to the degree of inflammatory infiltration to predict episodes of AGR accurately at early stages (18).

Classical major histocompatibility complex (MHC) class I and II molecules are known to play important roles in allograft rejection (20). In addition, nonclassical MHC class I molecules (such as HLA-G and Qa-2) have been well studied in the field of embryo implantation and development (4). Recently, HLA-G attracted attention in the field of transplantation. With limited polymorphism and immunotolerance, upregulated HLA-G expression in grafts and plasma has been reported to correlate with allograft acceptance (21,22).

Qa-2, the murine homolog of HLA-G, is encoded by the H2-Q7/Q9 gene pair (also known as Ped and Q9), which is located in the MHC region of chromosome 17 (16). Qa-2 has structural and immunological characteristics similar to those of HLA-G. Reportedly, Qa-2 can increase the rate of development of early embryos and assist in their survival (23), as well as protect tumor cells from NK cell– and lymphokine-activated killer cell–mediated cytolysis (24). However, the relationship of Qa-2 expression with immunosuppressive agents and graft rejection remains unexplored.

In our study, grafts and PBLs of group S mice showed negative or weakly positive Qa-2 expression compared with the control mice, suggesting that surgical stress had little influence on Qa-2 expression.

By contrast, Qa-2 expression in grafts and on PBLs of group SI mice was higher

than that in control or group S mice. These results suggest that the upregulation of Qa-2 can be attributed to the immunosuppressive treatments. In fact, Luque *et al.* reported that some patients treated with immunosuppressive agents exhibited increased soluble HLA-G (sHLA-G) expression after 2–4 hours of administration (25,26). The mechanisms of HLA-G upregulation by immunosuppressive agents are still unknown. Daniel *et al.* (27) reported that immunosuppressive agents such as cyclosporine A, methylprednisolone and mycophenolate mofetil can upregulate Th-2 cytokines (IL-4, IL-10, IL-12 and so on), which are able to induce HLA-G expression (28). Therefore, it is reasonable to speculate that immunosuppressive agents may increase Qa-2 expression on infiltrating lymphocytes and PBLs by upregulating the level of Th-2 cytokines.

The immunosuppressive treatment significantly prolonged the survival of the skin allografts, and this may be related to the increased expression of Qa-2. Under immunosuppressive treatment, Qa-2 might protect the transplants from rejection by inhibiting the function of cells involved in graft rejection, thereby prolonging allograft survival. Some studies have reported that HLA-G inhibits the cytotoxic activity of CD8^{+} T cells and NK cells (29), the proliferation of CD4^{+} T cells (30), the cell-cycle progression of alloreactive T cells (31) and the maturation of antigen-presenting cells (32). Some investigators found that HLA-G efficiently induced immunosuppressive T cells (33). In this study, we used a combination of cyclosporine A, prednisolone and mycophenolate mofetil to simulate a clinical immunosuppressive regimen; however, we did not determine which agent had the most potent effect in this process or the precise effective dosage. Further animal studies are necessary.

With respect to Qa-2 expression and the degree of AGR, downregulation of Qa-2 expression on infiltrating inflammatory cells and PBLs was observed as the lymphocyte infiltration steadily increased and

as AGR advanced, during which time the histology developed into grades 1 and 2. This result suggests that an inverse relationship between Qa-2 level and allograft rejection exists. Luque *et al.* (25) reported a similar relationship between sHLA-G and recurrent severe rejection. The mouse skin allograft model is considered to be the most difficult model to induce tolerance because of the dendritic and lymphocytic cells in the skin. In our study, the low doses of immunosuppressive agents used could only prolong the survival of the skin grafts, and they failed to completely inhibit the proliferation and activation of lymphocytes, as well as their severe infiltration into the graft. After the allografts were transplanted onto the recipients, the host T lymphocytes and monocytes (which constitute the majority of peripheral blood mononuclear cells [PBMCs]) were activated through direct and indirect recognition. If this activation is stronger than the inhibition due to immunosuppressants, it can lead to a rejection response manifested by the production of Th1-type cytokines, especially IL-2, interferon (IFN)-γ, and tumor necrosis factor (TNF)-α, as well as a change in the Th1/Th2 balance. These cytokines have been shown to downregulate HLA-G expression (34,35).

In the late stages of rejection, when the allograft began to display necrosis and slough off from the recipient, the inhibitory action of the immunosuppressive agents gradually gained an advantage over the activation of inflammatory cells and prevented the overproliferation of T cells to maintain the immune balance of the organism. At the same time, the expression of Th1-like cytokines decreased and Th2-like cytokines (IL-4, IL-10) were elevated (36), which may induce the expression of Qa-2. Our results showed that Qa-2 expression on PBLs increased gradually when rejection advanced into grade 3. At this stage, however, we failed to detect positive staining on the membrane or in plasma of infiltrating lymphocytes in the grafts because a large number of these cells began to be necrotic.

A time-dependent decrease in Qa-2 expression was observed at the early stages of AGR, much earlier than complete gross rejection. Immunological monitoring of the peripheral blood of transplant recipients is cheap and feasible. Thus, the decreased expression of Qa-2 on PBLs may provide important information for the clinical diagnosis of AGR. Serial detection of Qa-2 on PBLs may provide a means of predicting AGR episodes in a timely fashion.

In conclusion, we conducted a novel study on the time-dependent changes in Qa-2 expression in allografts and PBLs of recipients during the entire course of AGR. The expression of Qa-2 was upregulated by immunosuppressive treatment, but this subsequently decreased during allograft rejection. Serial monitoring of Qa-2 expression in PBL subsets after transplantation showed acceptable diagnostic values when used as a noninvasive sensitive early marker for the prediction of acute allograft rejection, as well as for monitoring the effects of immunosuppressive treatments.

DISCLOSURE

The authors declare that they have no competing interests as defined by *Molecular Medicine*, or other interests that might be perceived to influence the results and discussion reported in this paper.

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