Original Article

Changes in the Expression of Fas on T lymphocytes After Allogeneic Fetal Thymus Transplantation in Systemic Lupus Erytematosus Mice

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Abstract. *Background*: Systemic lupus erythematosus (SLE) is an autoimmune disorder that can produce inflammation in many organ systems. The rate of spontaneous apoptosis in lymphocytes from human SLE patients has been reported to be increased both in vivo and in vitro. Studies of fetal thymus transplantation in an autoimmune syndrome indicate that cellular immunity can be reconstituted and regulatory T cell functions can be normalized.
Objectives: The aim of this study was to assess changes in Fas expression on T lymphocytes following fetal thymus transplant.
Methods: (B/CxB6) F_1 (H-$2^{d/b}$) female F_1 mice were assigned to groups designated transplantation, normal, and control. Mice in the transplantation and control groups received parental BALB/c lymphocytes intravenously. Thirty days after injection of the lymphocytes, each F_1 mouse in the transplantation group received a fetal thymus graft under the right renal capsule. Mice in the control group did not receive thymus transplant and mice in the normal group received neither parental lymphocytes nor a fetal thymus graft. All mice received cyclosporin A at 2 mg/kg daily for the first 12 days after transplantation or starting on the corresponding day. Thirty days after thymus transplantation, F_1 mice were sacrificed and expression of Fas in peripheral blood lymphocytes was analyzed by flow cytometry.
Results: The percentage of $CD4Fas^+$ T lymphocytes was significantly increased in the control group and the transplantation group compared with the normal group. Corresponding significant differences were observed for $CD8Fas^+$, $CD4CD25Fas^+$, and $CD45RB^{low}Fas^+$ T lymphocytes.
Conclusions: In this study, we found that fetal thymus transplantation had a significant effect on the expression of Fas by T cell subtypes in SLE mice.

Key words: Lupus erythematosus, systemic. Thymus gland. Transplantation. Apoptosis. Fas.

Resumen. *Antecedentes*: El Lupus Eritematoso Sistémico (LES) es un trastorno auto inmune que puede inducir inflamaciones en varios órganos. Se ha confirmado el aumento en la tasa de apoptosis espontánea de linfocitos en pacientes con LES tanto in vivo como in vitro. Varios estudios sobre trasplante de timo fetal en casos de síndrome auto inmune indican la posible reconstitución de la inmunidad celular y la posible normalización de las funciones en las células T reguladoras.
Objetivos: El objetivo de este estudio es evaluar los cambios en la expresión Fas en linfocitos T previo trasplante de timo fetal.
Métodos: Se designaron varios grupos de ratones hembra F_1 (B/CxB6) F1 (H-$2^{d/b}$) para trasplante, normal y de control. Los ratones de grupo trasplante y de control recibieron linfocitos Balb/c parentales por vía intravenosa. 30 días después de la inyección de linfocitos Balb/c parentales, cada ratón F_1 del grupo trasplante recibió un injerto de timo fetal bajo la cápsula renal derecha. Los ratones en el grupo de control no recibieron trasplante de timo y el grupo normal no recibió ni linfocitos parentales ni injertos de timo fetal. A todos los ratones se les administró diariamente 2 mg/Kg de ciclosporina A durante los 12 primeros días posteriores al trasplante o empezando en el día correspondiente. Los ratones F_1 se sacrificaron 30 días después del trasplante de timo y se analizó la expresión Fas en linfocitos de sangre periférica a través de una citometría de flujo.
Resultados: El porcentaje de linfocitos $CD4Fas^+$ aumentó de manera significativa en los grupos de control y

trasplante comparado con el grupo normal. Se observaron diferencias significativas correspondientes en linfocitos $CD8Fas^+$, $CD4CD25Fas^+$, y $CD45RB^{bajo}$ Fas^+ T.
Conclusiones: A través de este estudio encontramos que el trasplante de timo fetal tiene efectos significativos en la expresión Fas por subtipos de células T en ratones con LES.

Palabras clave: Lupus Eritematoso Sistémico. Glándula del timo. Trasplante. Apoptosis. Fas.

Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disorder that can produce inflammation in many organ systems. Many lines of evidence from both human and animal studies suggest that T cells play a central role in the immune pathogenesis of SLE [1]. Data from studies in humans and mice have led to the widespread belief that T cells can support autoantibody-producing B cells in SLE [2]. Fas antigen (CD95) is known to be involved in apoptotic cell death and has also been implicated in the regulation of T cell tolerance [3]. Some studies have suggested that serum soluble Fas is involved in controlling lymphocyte apoptosis in SLE [4]. B cells produce autoantibodies against cell surface and nuclear antigens released by apoptotic lymphocytes and the production of these antibodies is closely linked to disease onset [5].

The involvement of the thymus in SLE pathogenesis provides clear evidence that it is an autoimmune disease [6]. Studies of fetal thymus transplantation in an autoimmune syndrome indicate that cellular immunity can be reconstituted and regulatory T cell functions can be normalized [7].

In this study, we analyzed changes in Fas expression on T lymphocytes after fetal thymus transplantation in SLE mice.

Materials and Methods

Animals and Reagents

Female BALB/c (B/C, H-2^d) and male C57BL/6 (B6, H-2^b) mice (6-8 weeks old) were purchased from the Bioproducts Insititute of Shanghai, China, and maintained in our animal unit. (B/CxB6) F_1 (H-$2^{d/b}$) female mice were mated in our animal unit. DBA/2 mice were provided by the Experimental Animal Research Center of the Fourth Military Medical University, Xi'an, China. All animal experiments were carried out, in our animal unit with the permission of the Experimental Animal Management Authority, Zhejiang, China.

Fluorescein isothiocyanate-labeled rat anti-mouse Fas monoclonal antibody (mAb), phycoerythrin (PE)-conjugated rat anti-mouse CD8a, CD25, and CD45RB mAb, and Peridinin-chlorophyll-protein complex (PerCP)-labeled rat anti-mouse CD4 mAb were purchased from PharMingen (San Diego, California, USA).

Animal Groups

F_1 mice were assigned to 3 groups designated transplantation, normal, and control; each group contained 6 mice. In the transplantation group, each mouse received (i) parental BALB/c lymphocytes intravenously, (ii) a fetal thymus graft under the right kidney capsule, and (iii) cyclosporin A at 2 mg/kg daily for the first 12 days after transplantation. In the normal group, mice only received cyclosporin A at 2 mg/kg daily for the first 12 days after the time of transplantation in the transplantation group and did not receive either parental lymphocytes or a fetal thymus graft. In the control group, each mouse received (i) parental BALB/c lymphocytes intravenously but no transplant, and (ii) cyclosporin A at 2 mg/kg daily for the first 12 days corresponding to the period following transplantation in the transplantation group. Details of these procedures are given in the following sections.

Preparation of Animal Models

Thymus, spleen, and lymph nodes were harvested from BALB/c mice, which were used as a source of lymphocytes. Tris-NH_4Cl buffer was added to destroy red blood cells. Lymphocyte suspensions were washed 3 times with Hanks' solution and trypan blue solution was added to identify live lymphocytes. In total, 5×10^7 parental BALB/c mouse lymphocytes were injected into each (BALB/c × C57BL/6)F_1 mouse in the transplantation and control groups by intravenous injection [8, 9]. Thirty days after the final injection, experimental SLE-like mice were successfully generated (concentrations of antinuclear antibodies [ANA] were analyzed by indirect immunofluorescence and direct immunofluorescence was used to analyze frozen sections of kidney; data not shown).

Thymus Transplantation

Thymus glands were taken from fetuses removed from pregnant DBA/2 mice at embryonic day 17 or 18. Thirty days after the preparation of the animal models (parental BALB/c lymphocyte injection), each F_1 mouse in the transplantation group received a fetal thymus graft under the right renal capsule. Cyclosporin A (Sandoz Research Institute, East Hanover, New Jersey, USA) was injected intraperitoneally at 2 mg/kg daily for 12 days in each mouse in each of the 3 groups [10].

Flow Cytometry

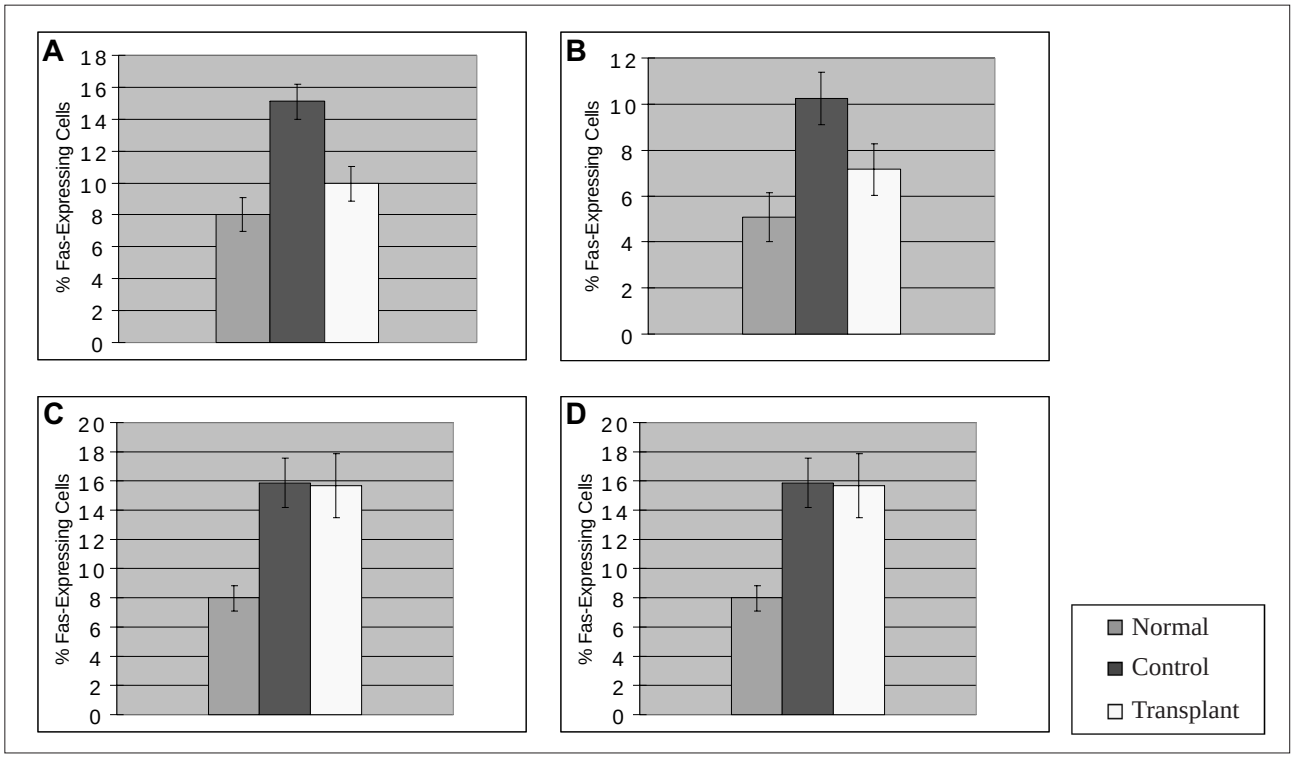
Thirty days after thymus transplantation, F_1 mice were sacrificed and their peripheral blood lymphocytes (PBL) obtained. T lymphocytes were separated from PBL using rat anti-mouse CD3 mAb immunomagnetic beads (Dynal, Oslo, Norway). Flow cytometry was performed on a FACScan flow cytometer using CELLQuest software (Becton Dickinson, New Jersey, USA). All T lymphocytes used in the experiment were prepared as a single cell solution with a density of 2×10^7 cells/mL. Cells were aliquoted into staining tubes at approximately 10^6 cells per tube. Three-color staining was then performed using PerCP-labeled anti-mouse CD4 mAb, PE-conjugated anti-mouse CD25 mAb, PE-conjugated anti-mouse CD8a mAb, PE-conjugated anti-mouse CD45RB mAb, and FITC-labeled anti-mouse Fas mAb. All mAbs were used at the concentrations recommended by the manufacturer. Each measurement was set to stop after 10 000 T lymphocyte events were acquired.

Statistical Analysis

Data processing and statistical analyses were performed using SPSS for Windows software version 10.0. Data are expressed as the mean ± SD. Variables were analyzed by one-way analysis of variance. *P* values less than .05 were considered significant.

Results

All mice were 15 to 16 weeks old when the data on Fas expression were obtained. The percentage of $CD4Fas^+$ T lymphocytes was significantly increased in the control group and the transplantation group compared with the normal group (15.11% ± 1.09% in the control group and 9.96% ± 1.09% in the transplantation group compared with 8.04% ± 1.05% in the normal group; $P < .01$ for both comparisons). Corresponding significant differences were observed for the following subsets of T lymphocytes: $CD8Fas^+$ (10.24% ± 1.14% in the control group and 7.15% ± 1.12% in the transplantation group versus 5.08% ± 1.07% in the normal group; $P < .01$ for both comparisons), $CD4/CD25Fas^+$ (29.88% ± 1.79% in the control group and 20.01% ± 1.92% in the transplantation group versus 14.68% ± 1.07% in the normal group; $P < .01$ for both comparisons), and $CD45RB^{low}Fas^+$ (15.89% ± 1.69% in the control group and 15.70% ± 2.20% in the transplantation group versus 8.00% ± 0.86% in the normal group; $P < .01$ for both comparisons).



Percentages of CD4 (A), CD8 (B), CD4CD25 (C), and $CD45RB^{low}$ (D) T cells expressing Fas in mice receiving parental lymphocytes followed by fetal thymus transplant, and cyclosporin treatment (transplant), parental lymphocytes and cyclosporin but no transplant (control), or cyclosporin alone (normal) (n = 6 in each group). T-cell subtypes expressing Fas were analyzed for 10 000 T lymphocytes in each group. Values are means and error bars indicate SD.

To determine whether thymus transplantation had a significant effect in the treatment of the female F_1 mice, we compared the percentages of CD4, CD8 and CD4/CD25 cells that were positive for Fas expression between the control group and the transplantation group. These percentages were significantly different in mice that had undergone thymus transplantation ($P < .01$), with the exception of the percentage of $CD45RB^{low}Fas^{+}$ T lymphocytes ($P > .05$), as shown in the figure.

Discussion

Fas, a 45-48 kilodalton cell-surface protein, is a member of the tumor necrosis factor/nerve growth factor receptor family [11, 12]. It is expressed on rapidly proliferating cells and its expression in lymphocytes has been reported to be strongly upregulated when they are activated [13]. Cross-linking of Fas by Fas ligand (FasL) leads to apoptosis [14]. Mutations in Fas and FasL are reported to be responsible for an autoimmune syndrome that leads to hypergammaglobulinemia, B cell defects, loss of T cell tolerance, and production of autoantibodies [15, 16]. However, alterations in the expression or function of Fas or FasL have not been clearly linked to SLE in humans. In-vitro analysis of apoptosis in lymphocytes from human SLE patients revealed a higher rate of spontaneous apoptosis compared with lymphocytes from healthy individuals or patients with rheumatoid arthritis [17, 18]. Furthermore, the rate of apoptosis in lymphocytes from patients with SLE has been reported to be elevated in comparison to healthy controls, both in vivo and in vitro [19].

Generation of H-2-incompatible F_1 mice can trigger the production of alloreactive donor T helper cells and induce a syndrome strongly resembling SLE [8, 20, 21]. The thymus exhibits significantly diminished function in SLE, particularly in untreated patients or patients who have active disease [22]. The thymus can act as the site of thymocyte apoptosis and apoptotic cell clearance, it may be involved in digestion of apoptotic cells, and is thought to be able to rapidly clear apoptotic T cells [23]. Fetal thymus transplants are not rejected [24] and the use of such transplants has been suggested for the treatment of numerous diseases [25].

In this study, F_1 model mice presented a significant decrease in the number of T-cell subsets —including CD4, CD8, and CD4CD25 positive T lymphocytes—that expressed Fas following thymus transplant. While the exact mechanism is unclear, we deduced that the Fas-expressing CD4 or CD8 T lymphocytes underwent a process of negative selection in the transplanted thymus. Such a process might induce autoreactive T lymphocyte anergy, mostly through the Fas-FasL pathway. Chimerism within the receptors could also be involved in the inhibition of T cell proliferation [26]. In addition, cytokine imbalance has been reported in both SLE patients and experimental models of the disease [27, 28]. It is possible that cytokines and thymus hormones released from the graft played a role in correcting the cytokine imbalance. Nevertheless, further studies will be needed to address the exact mechanisms.

Interestingly, we observed that there was no statistical difference in the percentages of $CD45RB^{low}Fas^{+}$ T-cell subsets between the control group and the transplantation group. In humans, the memory subset of CD4 and CD8 T cells is defined by reactivity with the anti-CD45RO mAb UCHL1, and is thus designated the CD45RO subset [29]. In mice, the memory subset is defined by low reactivity to an anti-CD45RB-isoform mAb, and is designated CD45RBlo [30, 31]. Part of the reason for the absence of a statistically significant difference between the control group and the transplantation group is presumed to be the higher level of antiapoptotic molecules, such as bcl-2, expressed on $CD45RB^{low}$ memory T cells than on effectors. Thus, effector and memory T cells may differ in susceptibility to apoptosis [32].

In conclusion, we analyzed changes of Fas expression on T lymphocyte subsets after thymus transplantation in SLE mice and found that allogeneic grafts significantly altered expression of Fas on T cell subtypes.

Acknowledgments

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