The Role of the Thymus in Development of Necrotizing Arteritis in Transgenic Rats Carrying the env-pX Gene of Human T-Cell Leukemia Virus Type-I

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Necrotizing arteritis mimicking polyarteritis nodosa occurred in transgenic rats carrying the env-pX gene of human T-cell leukemia virus type I. To investigate the pathogenesis of necrotizing arteritis in these rats (env-pX rats), adoptive transfers of spleen cells and bone marrow cells were done from env-pX rats before they developed arteritis to nontransgenic rats. Necrotizing arteritis occurred in lethally irradiated nontransgenic rats reconstituted by env-pX spleen cells, thus indicating that the env-pX transgene in affected vessels may not be essential for the development of arteritis. In contrast, arteritis was not induced in nontransgenic recipients by adoptive transfers of env-pX bone marrow cells, which suggested that T cells derived from the env-pX thymus may play a role in the development of arteritis. To clarify if the process of differentiation of T cells in the env-pX thymus is crucial to develop necrotizing arteritis, reciprocal exchange of thymus frameworks was done between env-pX and nontransgenic rats. Necrotizing arteritis occurred in nontransgenic rats with an env-pX thymus framework, whereas development of arteritis was suppressed in env-pX rats in which the thymus framework was replaced with a nontransgenic one. This collective evidence shows that the thymus is directly associated with the development of necrotizing arteritis in env-pX rats.

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Materials and Methods

Rats

Inbred WKAH rats and WKAH rats bearing the env-pX gene of HTLV-I (env-pX rats) were maintained at the Institute for Animal Experimentation, Hokkaido University Graduate School of Medicine. Experiments on animals were done in accordance with the Guide for the Care and Use of Laboratory Animals in Hokkaido University Graduate School of Medicine (http://www.hokudai.ac.jp/animal/houki/hokudaisisin.html).

Polymerase Chain Reaction (PCR) for the Transgene

Peripheral blood mononuclear cells were separated from total blood of rats, using Lympholyte Rat (Cedarlane, Ontario, Canada). The env-pX transgene in genomic DNA extracted from the peripheral blood mononuclear cells was amplified by PCR, as described.

Cell Transfer Experiments

Mononuclear cells were separated from spleen and bone marrow, respectively, using Lympholyte Rat. Donor env-pX rats (male, 6 to 8 weeks of age) were confirmed microscopically to be disease-free. Nontransgenic WKAH rats (male, 6 weeks of age) were lethally irradiated at 12 Gy by 60Co and served as recipients. SCs or BMCs from env-pX rats were injected via the tail vein of lethally irradiated WKAH rats. Each recipient was given 1/10^7 SCs or BMCs from a single donor. Transfers from env-pX to env-pX and from WKAH to WKAH rats were made to serve as positive and negative controls, respectively.

Reciprocal Exchange of Thymus Frameworks—Thymectomy and Thymic Transplantation and BMC Transfer (Tx+TT+BMT)

Thymi from newborn env-pX rats were physically crushed to remove >90% of the thymocytes, then the residual thymic tissues were soaked in RPMI 1640 medium containing 1.5 mmol/L of 2-deoxyguanosine (Sigma, St. Louis, MO) for 7 days, as described by Martin-Fontecha and colleagues. After the deoxyguanosine treatment, histological examinations showed that the thymus frameworks remained intact and most of the residual thymocytes were critically damaged (data not shown). The thymus frameworks were implanted into the renal subcapsular space of WKAH rats (male, 6 weeks of age) that had been given lethal irradiation (12 Gy) followed by thymectomy. Then, hematopoietic cells of these rats were reconstituted by WKAH BMCs (1 × 10^7 BMCs from a single donor/recipient). A reverse combination of env-pX and WKAH rats was also done.

Analysis of the Implanted Thymus Framework

To examine reconstitution of the thymus, recipients were sacrificed 2 months after the Tx+TT+BMT. The kidney implanted with the thymus framework was sectioned and stained with hematoxylin and eosin (H&E). In addition, phenotype of lymphocytes in the reconstituted thymus was analyzed using flow cytometry. For this purpose, cells were stained with fluorescein isothiocyanate-labeled anti-rat CD4 and phycoerythrin-labeled anti-CD8 antibodies (Pharmergen, San Diego, CA).

Evaluation of Vascular Lesion

Six months after the cell transfer and Tx+TT+BMT experiments, recipients were sacrificed for pathological examinations. Systemic organs, including cerebrum, cerebellum, spinal cord, salivary glands, heart, lung, liver, kidney, adrenal glands, pancreas, intestine, testis, thymus, spleen, lymph nodes, ankle joints, muscle, and skin were sectioned and stained with H&E. Specimens with evident vascular lesions were further examined in elastica van Gieson, Masson trichrome, and phosphotungstic acid hematoxylin stainings, and in immunohistochemistry using anti-rat CD3 antibody (Cedarlane).

Laser-Capture Microdissection

From formalin-fixed paraffin-embedded sections (4 μm), 1 × 10^7 lymphocytes accumulating at the affected vessels were dissected using Laser Capture Microdissection LM2000 (Arcturus, Mountain View, CA). An equal number of env-pX lymph node cells were microdissected similarly and served as a positive control.

Nested PCR

DNA was extracted from the microdissected samples. To detect the pX gene, nested PCR was done using oligonucleotide primers, 5′-GCATGACACAGGCAAGCAT-3′ (sense) and 5′-CCGAACATAGTCCCCAGAGAT-3′ (antisense) for outer primers (35 cycles), and 5′-CAGATA-CAAAATTTAACCATGCTTATTATCA-3′ (sense) and 5′-ACACATGACTGGGTATCCGAAA-3′ (antisense) for inner primers (40 cycles). The products were electrophoresed and analyzed using Bioanalyzer (Agilent Technologies, Waldbronn, Germany).

Table 1. Summary of Cell Transfer Experiments

<table>
<thead>
<tr>
<th>Donor rats</th>
<th>env-pX</th>
<th>WKAH</th>
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<tbody>
<tr>
<td>Recipients rats</td>
<td>WKAH</td>
<td>WKAH</td>
</tr>
<tr>
<td>Cells transferred</td>
<td>SC</td>
<td>BMC</td>
</tr>
<tr>
<td>SC</td>
<td>BMC</td>
<td></td>
</tr>
<tr>
<td>Necrotizing arteritis*</td>
<td>3/9</td>
<td>0/9</td>
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</tbody>
</table>

*Rats with necrotizing arteritis/number of rats histologically examined.
Results

Development of Necrotizing Arteritis in Lethally Irradiated WKAH Rats Reconstituted by env-pX SCs

Results of cell transfer experiments are summarized in Table 1. Necrotizing arteritis was evident in three of nine lethally irradiated WKAH rats (33%) that underwent SC transfers from env-pX rats. The affected vessels included arterioles in the mediastinum and testicular arteries. The arteritis was of the necrotizing type and similar to naturally occurring arteritis in env-pX rats (Figure 1). Additionally, the incidence of arteritis was equivalent to that in untreated env-pX rats (9 of 27, 33%).18 No evidence of arteritis was found in nine lethally irradiated WKAH rats that received env-pX BMCs.

Immunohistochemical study revealed that the infiltrating cells included CD3+ T cells (Figure 1D). SCs contain heterogeneous cell populations. Among them, one major
The Thymus Is Crucial for Necrotizing Arteritis to Occur in env-pX Rats

To clarify whether the env-pX thymus plays an essential role in the pathogenesis of necrotizing arteritis, reciprocal exchange of thymus frameworks (Tx/T.T+T+BMT experiment) was done between env-pX and WKAH rats (see Materials and Methods). Two months after the Tx/T.T+T+BMT, histological examination revealed that cortical and medullary structures of the thymus had been reconstituted in the renal subcapsular space where the thymus framework had been grafted (Figure 2A). Because the majority of lymphocytes in this reconstituted thymus were positively stained for both CD4 and CD8 (Figure 2B), the heterotopically grafted thymus framework was considered to be functioning in the recipients. When genomic DNA extracted from the peripheral blood mononuclear cells of recipient WKAH rats with an env-pX thymus graft was examined to identify contamination of env-pX lymphocytes derived from the implanted thymic tissue, the env-pX transgene was not detected by PCR (Figure 2C). Thus, the level of contamination was considered to be below the sensitivity of our PCR (<1 env-pX lymphocyte per 10⁴ normal lymphocytes).

At 6 months after the Tx/T.T+T+BMT, 4 of 11 WKAH rats (36%) with an implanted env-pX thymus framework showed evidence of development of necrotizing arteritis (Table 2). Histopathological features and the incidence were similar to those of necrotizing arteritis seen both in env-pX rats and WKAH rats that received adoptive transfers of env-pX SCs (Figure 3). The env-pX transgene was not detected in a DNA sample extracted from lymphocytes microdissected from the arteritis lesion by nested PCR (Figure 3D). Arteritis never occurred in env-pX rats in which the thymus framework was replaced with a nontransgenic one.

Discussion

Vasculitides in humans vary in pathological characteristics, suggesting that diverse etiologies may be implicated in the syndromes. Analysis of animal models facilitates
understanding of the pathogenesis. F1 mice of New Zealand Black and White strains (NZB×NZW F1) develop glomerulonephritis at a high frequency and also necrotizing arteritis albeit with a lower frequency.24 Because deposition of immune complexes and complements is evident in affected glomeruli and vessels, humoral immune responses play direct and crucial roles in the pathogenesis. NZB×NZW F1 mice are regarded as being a good model for glomerulonephritis and necrotizing arteritis related to systemic lupus erythematosus in humans. On the other hand, deposition of neither immunoglobulins nor complements in affected vessels may be evident in some cases of vasculitis, including polyarteritis nodosa, hence other factors than immune complex-mediated inflammation may be involved. Necrotizing arteritis in env-pX rats mimicking polyarteritis nodosa in humans also seems to be irrelevant to the pathogenetic immune complexes, because only IgM but not IgG or complements is deposited in affected vessels and distributed IgG suggesting a nonspecific permeation is evident around the lesion (data not shown).

Because necrotizing arteritis similar to that seen in env-pX rats occurred in nontransgenic rats given adoptive transfers of env-pX SCs, the env-pX transgene in affected vessels may not be essential for the development of arteritis. In addition, based on findings that the infiltrating cells contained T cells and that adoptive transfers of env-pX SCs but not BMCs induced necrotizing arteritis in nontransgenic rats, we assumed that T cells derived from the env-pX thymus may play an important role in the development of necrotizing arteritis in env-pX rats. Results of reciprocal exchange of the thymus frameworks between env-pX and nontransgenic rats (Tx/H11001TT/H11001BMT experiment) clearly showed that our hypothesis is tenable. Sumita and colleagues25 established a T cell clone that recognized rat vascular smooth muscle cells from MRL+/+ mice, and they demonstrated that an adoptive transfer of these T cells induced pulmonary vasculitis in MRL-+/+ mice.

### Table 2. Summary of Reciprocal Exchange of Thymus Frameworks

<table>
<thead>
<tr>
<th></th>
<th>BMC donor rats</th>
<th>Thymus donor rats</th>
<th>Recipient rats</th>
<th>Necrotizing arteritis*</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKAH env-pX</td>
<td>env-pX WKAH</td>
<td>env-pX WKAH</td>
<td>4/11</td>
<td>0/11</td>
</tr>
</tbody>
</table>

*Rats with necrotizing arteritis/number of rats histologically examined.

![Figure 3](image-url)  
Figure 3. Necrotizing arteritis in a WKAH rat with an env-pX thymus framework. The affected vessel in this figure is the testicular artery (original magnification, ×200). A, H&E staining; B, elastica van Gieson staining (disruption of elastic fibers is evident (arrows)); C, phosphotungstic acid hematoxylin staining (fibrinoid degeneration of the arterial wall is evident (arrow)); D, detection of the pX gene in DNA extracted from microdissected samples by nested PCR. Lane 1, ladder marker; lane 2, sample from env-pX lymph node cells as positive control; lane 3, sample from lymphocytes accumulating at the arteritis lesion in a WKAH rat with an env-pX thymus framework. An expected molecular size of the nested PCR product is 90 bp.
env-pX SCs may possibly contain autoreactive T cells that recognize vascular components unrelated to the env-pX transgene, although SC donors at the time of transfer experiments did not manifest arteritis. Further investigations are needed to clarify which population of SCs is directly associated with the development of necrotizing arteritis in env-pX rats, and to identify the target molecule(s) of vascular components recognized by these cells.

Because the env-pX transgene was not detected in the arteritis lesion of a Tx+TT+BMT recipient, it seems unlikely that contaminated cells derived from the env-pX thymic tissue accumulated or expanded at the lesion. It is considered that nontransgenic T cells reactive with self-vascular components may be generated or may not be deleted with an interaction with the env-pX thymus framework. Autoreactive T cells are eliminated through positive deletion with an interaction with the env-pX thymus framework; M. Ishizaka for secretarial services.

Acknowledgments

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References

22. Martin-Fontecha A, Broekhuizen R, de Heer C, Zapata A, Schuurman HJ: Transplantation of cultured thymic fragments in congenitally athymic and euthymic rats. Culture with deoxyguanosine or cyclosporin A


