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Premature senescence of T cells in long-term survivors of renal transplantation

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ABSTRACT

Prevention of graft rejection in renal transplant recipients depends on chronic treatment with immunosuppressive agents. However, impaired immune functions and immunosurveillance may cause infection, cancer and many other problems, which subsequently compromise quality of life and survival of patients. In the present study, we assessed potential premature immune-senescence in long-term survivors of kidney transplant patients receiving immunosuppressive agents. Peripheral blood lymphocytes derived from patients had significantly shorter telomeres than those from age- and sex-matched healthy individuals. Consistent with this, lower expression of telomerase reverse transcriptase (hTERT) and telomerase activity was observed in patients' lymphocytes. The level of $p16^{ink4A}$ expression was elevated in patients' cells. Moreover, the $CD8^+/CD28^-$ fraction of late-stage differentiated T cells was significantly increased in the patients. *In vitro* studies further showed that cyclosporine A, a widely used immunosuppressive drug in transplant patients, attenuated induction of hTERT and telomerase activation in T cells treated with the mitogenic agent concanavalin A. Taken together, immunosuppressant-mediated premature senescence of T lymphocytes occurs in renal transplant recipients.

1. Introduction

Kidney transplantation has become the therapy of choices for the patients with end-stage renal disease, and the introduction of new potent immunosuppressive drugs targeting T lymphocytes during the last decade has led to a dramatic decrease in graft rejection, thereby offering an increased quality of life, survival and longevity [1–4]. However, long-term over-immunosuppression causes infection, malignancies and many other complications, which in turn compromises patients' quality of life and survival [2,5]. Therefore, there is a great need to develop methods for prevention of unnecessary or over-treatment with immunosuppressants.

With aging, a gradual deterioration of the immune system occurs, the so-called immunosenescence, which broadly affects both innate [natural Killer (NK) cells, macrophages, dentritic cells (DCs), and complement factors] and adaptive (T and B cells) immune components [6,7]. The resulting failure to mount effective immune responses confers aged individuals more susceptible to infections and more prone to develop cancers [6–8]. In addition, a premature aging of the immune system may also happen under certain circumstances such as the continuous challenge of the unavoidable exposure to a variety of antigens and harmful factors [9–12], which can be independent of aging. One of the most significant effects of aging on the immune system is impaired T lymphocyte development and function, which include the declined generation of new T cells, the accumulation and the clonal expansion of memory and effector T-cells, impaired proliferation to antigen stimulation, deregulation of intracellular signal transduction capabilities, attenuated capacity to produce effector lymphokines, etc. [6,7]. Biochemically, these senescent T cells harbor shorter telomeres, special DNA–protein structure at the termini of each chromosome that controls cellular life-span, and express lower telomerase activity, a RNA-dependent DNA polymerase responsible for telomere elongation [10,13,14].

As described above, T cells are the major targets of immunosuppressive agents for organ transplant recipients. However, it is currently unclear whether long-term administration of these agents triggers senescence of T cells. In the present study, we address this issue by analyzing lymphocytes derived from renal transplant recipients.

Abbreviations: ConA, concanavalin A; CysA, cyclosporine A; hTERT, human telomerase reverse transcriptase; LCs, lymphocyte counts; WBCs, white blood cells.

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2. Materials and methods

2.1. Patients and healthy controls

A total of 54 patients (male, 36 and female, 18) receiving kidney transplantation at the Shandong University Second Hospital were included in the study. Patients who had survived at least 4 years after the transplantation (from 4 to 12.5 years, and median 5.5 years) were selected and the median age of the patients was 48 years (range 25–68). The clinical information of the patients was listed in Table 1. Age- and sex-matched healthy individuals served as controls. Patients' and controls' peripheral blood was collected, and lymphocytes were isolated as described previously. The study was approved by the Local Ethics Committee.

2.2. RNA extraction and qRT-PCR

ULTRASPEC™-II RNA kits (Biotecx Laboratories, Houston, TX) or Trazol kits were used for isolation of the total RNA from blood samples and T cells. cDNA was synthesized using random primers (N6) (Pharmacia, Uppsala, Sweden). The sequences of the PCR primers were: hTERT: 5′-CGGAAGAGTGTCTGGAGCAA-3′ (forward) and

Table 1
Patients' characteristics, immunosuppressant use and WBC[a]/LC[b] counts.

Patient characteristics			Total amounts of immunosuppressants used					WBC	LC
No.	Age (y)/sex	Years	CsA (g)	AZP (g)[c]	MMF (g)[c]	FK-506 (mg)	Pred (mg)[c]	($\times 10^9$/L)	
1	56/F	5.4	346	91	0	548	9875	5.2	2.1
2	57/F	5.3	287	73	365	0	9575	7.1	2.9
3	58/F	7.1	0	0	3932	3833	9125	4.8	1.6
4	28/M	4.9	492	164	0	0	10,300	7.0	0.8
5	47/M	4.3	183	0	2325	2050	0	NA[d]	NA
6	48/M	4.7	283	30	1643	0	8500	4.2	1.6
7	42/M	4.8	280	0	2163	0	8650	4.7	1.5
8	46/M	4.8	392	110	831	0	8800	4.2	1.2
9	50/F	6.9	441	55	275	0	12,600	11.1	1.1
10	63/F	5.2	236	0	2356	0	14,900	11.3	1.1
11	37/M	6.1	499	102	275	0	11,100	4.7	2.1
12	36/M	6.2	394	0	3375	0	7900	5.4	1.8
13	53/M	8.2	745	18	0	0	29,800	7.5	1.7
14	52/M	9.8	645	161	365	0	17,925	4.0	0.3
15	57/M	5.3	350	0	3830	0	5700	6.1	1.9
16	54/M	4.3	343	79	1095	0	7900	7.0	2.1
17	48/M	6.9	103	100	3008	1545	12,600	NA	NA
18	54/M	7.2	373	186	0	2250	13,075	6.4	2.3
19	53/F	8.0	438	0	2920	0	14,600	3.2	1.3
20	39/F	4.0	237	73	0	0	7300	8.0	2.5
21	56/F	6.9	504	0	0	0	15,624	4.2	0.8
22	48/M	8.0	611	73	2190	0	18,250	9.3	2.0
23	49/F	4.0	329	128	275	0	7300	4.4	0.9
24	33/M	7.0	478	256	0	732	12,775	4.6	1.1
25	43/M	7.4	511	0	730	63	22,359	9.7	1.8
26	42/M	6.3	438	114	0	21	11,406	5.8	1.3
27	68/M	9.0	433	0	548	0	16,425	5.2	0.8
28	42/F	7.0	438	128	0	0	12,775	6.2	1.8
29	57/M	5.1	0	0	2491	6493	4638	7.1	2.8
30	39/M	4.5	329	0	2464	0	8213	6.3	2.2
31	63/F	5.2	189	0	2828	0	4713	8.0	3.0
32	37/M	4.0	228	0	2190	0	7300	7.3	2.7
33	46/F	5.8	315	0	314	0	10,494	7.8	0.5
34	30/M	5.8	416	104	45	90	10,553	9.8	1.6
35	35/M	4.3	426	0	2325	0	7750	6.5	2.3
36	40/M	6.5	502	0	4152	913	11,863	8.0	0.8
37	62/M	12.5	698	18	0	2920	22,813	3.3	1.2
38	48/M	4.0	384	0	2555	0	7300	7.6	2.2
39	25/F	4.2	266	0	2280	0	3800	5.0	1.4
40	59/M	4.3	0	0	2370	2903	7900	4.5	1.8
41	49/M	9.0	666	73	2738	0	20,075	8.4	1.6
42	51/M	6.8	219	73	2163	810	12,300	3.8	0.8
43	55/M	5.1	0	0	2783	11,130	9275	6.0	0.9
44	63/M	4.0	0	0	1460	5110	7300	5.8	1.4
45	47/M	4.0	183	0	2190	2920	7300	8.0	2.1
46	62/F	4.4	242	0	2415	0	8050	5.9	2.8
47	57/F	7.1	485	0	3878	0	12,925	5.9	1.7
48	61/F	4.1	186	0	1673	0	7450	5.2	1.6
49	46/M	5.3	681	97	0	0	9725	8.3	2.4
50	60/M	7.2	0	0	5230	9153	13,075	6.3	1.8
51	46/M	6.0	0	0	6570	0	10,950	5.4	1.6
52	46/M	7.3	0	0	4264	0	9581	7.4	2.0
53	46/F	5.4	198	0	1481	0	9875	7.9	2.2
54	40/F	4.9	358	73	248	0	8950	6.2	1.3

[a] WBC, white blood cells.
[b] LC, lymphocytes.
[c] AZP, azathioprine; Pred, prednisone; MMF, mycophenolate mofetil.
[d] NA, not available.