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T-cell independent production of salivary secretory IgA after hematopoietic stem cell transplantation in children

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This study examined the recovery of secretory IgA (S-IgA) in saliva after hematopoietic stem cell transplantation (HSCT) in 35 children and young people between the ages of 3 and 27 years (mean = 13.6), and compared this recovery with that of serum immunologic constituents. Reference values for human salivary S-IgA in saliva were obtained from 77 healthy control subjects between the ages of 7 and 25 years (mean = 11.4). In the 35 patients, a nadir of secretory IgA concentrations in saliva (S-IgA) was observed between the 3rd and the 4th month, and a return to normal values 1 year after HSCT. Serum IgA concentrations reached their nadir in the 6th month, and normalized in the 18 months after HSCT. The recovery of T-helper cells ($CD4^+/3^+$) was also delayed to beyond 18 months. We found a significant correlation between the reconstitution pattern of S-IgA and that of T-helper lymphocytes, but no correlation was found between the post-transplant evolutions of S-IgA and serum IgA, or between S-IgA and T-helper cells. The recovery of S-IgA was more rapid than that of serum IgA and appeared to be T-helper cell independent.

Key words: hematopoietic stem cell transplantation; immune reconstitution; immunoglubulin A; secretory IgA; T-helper cells

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Hematopoietic stem cell transplantation (HSCT) is an established treatment for leukemia, lymphomas, solid tumors, and several diseases of the hematopoietic and autoimmune systems (18, 21–23, 29, 38). Infections, often of oral origin, are an important cause of morbidity and mortality at the time of transplantation, in part because of a weakened immune defense of the oral mucosa, in particular a decrease in the concentration of S-IgA (6, 12, 14, 28, 40). Most of the information in this field has been collected in adult patients and adult control subjects, and no data are available regarding the reconstitution of the immune defense after HSCT in the young (6–9, 40, 41). Furthermore, several studies in mice have suggested that the production of S-IgA may be T-cell independent (3, 16, 36). The aim of this study was to further examine whether the production of S-IgA in humans is independent of T-helper cells. In addition, since the concentrations of S-IgA in healthy children have rarely been reported, our study included a control population of 77 children and adolescents.

Material and methods

Study and control groups

The study group consisted of 35 patients between the ages of 3 and 27 years (mean = 13.6) who underwent HSCT for hematologic ($n = 24$) and nonhematologic diseases ($n = 11$) at the Children's Hospital of the University of Jena, Germany. There were 14 autologous and three allogeneic peripheral blood stem cell transplantations, and 17 allogeneic and one autologous bone marrow transplantation. The baseline characteristics of the patient population, the transplantation procedures, conditioning regimens and graft-vs.-host disease prophylaxis are presented in Table 1.

The control group included 77 healthy subjects between the ages of 7 and 25 years (mean = 11.4), screened from an initial population of 169 individuals who completed a questionnaire to exclude

Table 1. Baseline characteristics of the patient population, transplantation procedures, conditioning regimens and graft-vs.-host disease prophylaxis

Patient No.	Sex/Age	Diagnosis	Transplantation/ type/conditioning regimen	GvHD prophylaxis	Acute GvHD Grade/Localization	Chronic GvHD Localization
1	M/18	AML M4	M/allo/CPM, FTBI	CSA +MTX +PRED	4/skin	skin
2	M/20	T-ALL	B/auto/VP16, FTBI	CSA (GvL provocation)		
3	M/24	T-ALL	M/allo/ALG, Thiotepa, CPM, FTBI	CSA	0	
4	M/8	PNET	B/auto/L-PAM, VP16, FTBI			
5	F/6	Clear cell sarcoma kidney	B/auto/L-PAM, VP16, FTBI			
6	F/9	Rhabdomyosarcoma	B/auto/L-PAM, VP16, CARBO			
7	F/13	Ewing's sarcoma	B/auto/L-PAM, VP16, FTBI			
8	M/19	Osteosarcoma	B/auto/L-PAM, VP16			
9	M/14	Ewing's sarcoma	B/auto/L-PAM, VP16, FTBI			
10	M/16	AML-M1	M/allo/VP16, CPM, FTBI	CSA +MTX +PRED	0	
11	F/9	pre-B-ALL	M/allo/VP16, FTBI	CSA +MTX	0	
12	F/14	CML	M/allo/CPM, FTBI	CSA +MTX +HD-PRED	4/skin, liver, bowel	
13	M/12	B-NHL	B/allo/CPM, ALG, FTBI	CSA +MTX +HD-PRED	0	
14	F/13	Hodgkin's disease	B/auto/BCNU, CAR, VP16, L-PAM			
15	F/4	T-ALL	M/allo/VP16, FTBI	CSA +MTX +PRED	0	
16	F/9	Neuroblastoma	B/auto/L-PAM, P16, CARBO			
17	M/20	c-ALL	M/allo/ALG, VP16, FTBI	CSA +MTX +HD-PRED	3/skin, bowel	skin
18	M/16	AML M2	M/allo/BU, CPM, ALG	CSA +MTX +HD-PRED	2/skin	skin, liver
19	M/10	pre-B-ALL	B/allo/VP16, ALG, FTBI	CSA +MTX +HD-PRED	2/bowel	
20	F/3	Neuroblastoma	B/auto/L-PAM, CARBO			
21	M/16	Hodgkin's disease	B/auto/BCNU, CAR, VP16			
22	F/12	Rhabdomyosarcoma	B/auto/L-PAM, CARBO, VP16			
23	F/17	Ewing's sarcoma	B/auto/L-PAM, VP16			
24	M/22	Nephroblastoma	M/auto/VP16, CARBO, L-PAM			
25	M/9	ALL	B/allo/VP16, ALG, FTBI	CSA		
26	M/27	CMML	M/allo/BU, CPM, L-PAM	CSA +MTX +HD-PRED	0	
27	F/14	CML	M/allo/BU, CPM	CSA +MTX	1/skin	
28	F/10	pre-B-ALL	M/allo/ATG, VP16, FTBI	CSA +MTX +HD-PRED	0	skin, liver
29	M/16	CML	M/allo/BU, CPM, ATG	CSA +MTX	4/skin, liver, bowel	
30	M/7	CMML	M/allo/BU, CPM, L-PAM, ATG	CSA +MTX	1	
31	F/17	AML-M5	M/allo/BU, CPM	CSA +MTX	0	
32	M/13	MDS	M/allo/BU, CPM, L-PAM, ATG	MTX +PRED	4/skin, liver, bowel	
33	F/11	SAA	M/allo/–	CSA +MTX	1/skin, bowel	
34	F/15	CML	M/allo/CPM, ATG, FTBI	CSA +MTX	0	
35	M/14	AML M4	B/auto/BU, CPM			

ALG, antilymphocyte globulin. allo, allogeneic. auto, autologous. B, peripheral blood stem cell transplantation. BCNU, carmustine. BU, busulfane. CAR, cytarabine. CARBO, carboplatin. CPM, cyclophosphamide. CSA, cyclosporin A. FTBI, fractionated total body irradiation. GvHD, graft vs. host disease. GvL, graft vs. leukemia effect. HD-PRED, high-dose prednisone. L-PAM, melphalan. M, bone marrow transplantation. MTX, methotrexate. PRED, Prednisone. VP16, Etoposide.

those with allergic disorders, acute or chronic respiratory diseases, tonsillar hypertrophy, lesions of the oral mucosa, and active or passive smokers. All participants of this study, or their legal representatives, had granted their written informed consent.

Collection and handling of saliva specimens

In the morning just after the study participant had awakened, and before any food ingestion or oral or dental care, samples of unstimulated whole saliva were collected in patients and in control subjects, using a small, compressed cylinder of cotton/wool (Salivette^{TM} no. 51.1534, Sarstedt, Nümbrecht, Germany) kept in a sublingual position. The swabs were centrifuged for 4 min at 2767 **g**, then frozen to −20°C until analysis, which was performed within <6 months. The compliance of patients and healthy subjects was verified by nurses or parents, who supervised the sampling procedure, and who labeled and dated all samples. Improperly collected specimens were discarded.

Monitored parameters and methods of analysis

The concentrations of S-IgA were measured by radial immunodiffusion (17, 37) using a kit (No. RN148.3, The Binding Site, Birmingham, England) containing plates with monospecific antibodies against the human whole S-IgA dimer in an agarose gel. The agarose gel plates were incubated for 96 h with 10 µl of defrosted patient saliva at 22°C. A radial immunodiffusion plate reader was used to measure the diameters of the precipitin rings to the nearest 0.1 mm. The S-IgA concentrations were evaluated graphically, using three S-IgA calibrator samples for each plate. The minimal measurable concentration for S-IgA was 16.8 mg/l.

Secretory IgA was standardized by the manufacturer by measurement of the optical density (using E 1%/1 cm = 13.8 at 280 nm) of pure S-IgA from human milk, obtained by column chromatography. The purity of S-IgA was verified by the presence of a single line by immunodiffusion techniques, using antibodies against whole serum, IgA, secretory piece, and S-IgA, and by SDS PAGE.

The presence, on SDS page, of three bands at molecular weights of 70 kDa, 54 kDa, and 23 kDa, corresponding to the secretory component, alpha chain, and light chain, respectively, indicated that the S-IgA preparation was homogeneous. The concentrations of serum IgA were measured by rate nephelometry (Beckman Array Protein System, Beckman Coulter Instruments, Krefeld, Germany) using 20 µl of patient serum, in conjunction

with specific monoclonal antibodies against human IgA, according to the manufacturer's instructions (Beckman Immunochemistry Systems). The lowest detectable serum IgA concentration was 0.063 g/l.

The T-helper cells were analyzed using two-color flow cytometry (13) (marker CD3-FITC and CD4-PE, Dako, Hamburg, Germany; EPICS Profile II, Beckman Coulter) after antibody incubation of 100 µl of peripheral blood anticoagulated with EDTA for 15 min at room temperature, and subsequent lysis of the red blood cells by a Coulter Q-Prep procedure (Beckman Coulter). Usually, 10,000 lymphocytes were analyzed and, during the early post-transplant period, no fewer than 3000 cells were counted.

Blood and saliva were collected monthly for the first 6 months, and at 9, 12, and 18 months after HSCT. The last measurement was made 772 days after HSCT.

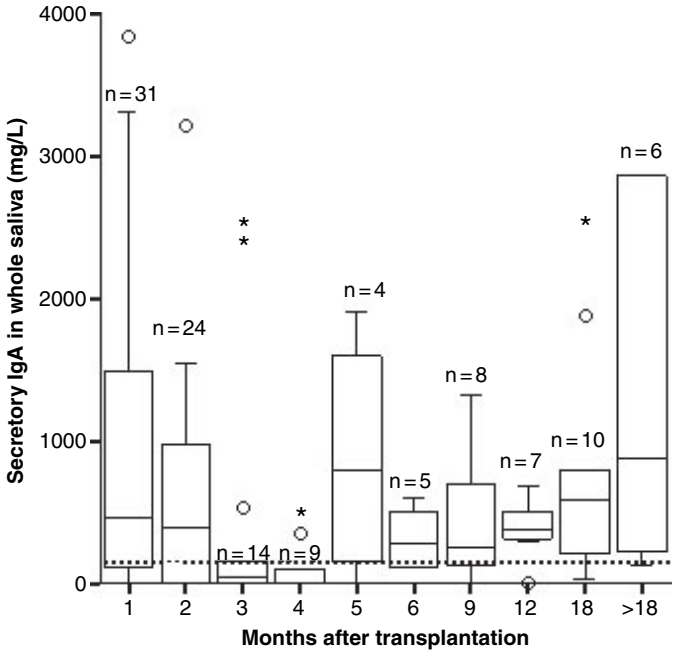

Fig. 1. Time course of secretory IgA in whole saliva after HSCT. Dotted line = lower level of normal range for mean age of all patients (13.6 years); o = outliers; * = extremes.

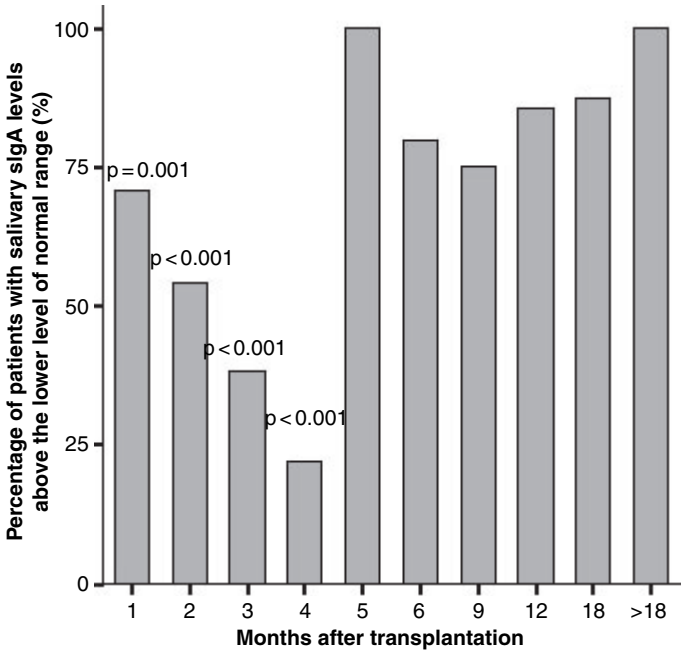

Fig. 2. Reconstitution of secretory IgA in whole saliva after HSCT with regard to normal range. Significance level for difference of distribution between normal population and patients is indicated (binomial test).

Statistics

Control group

The Gaussian pattern of the S-IgA distribution was tested with the Lilliefors test. Age-related differences were examined by the Mann–Whitney *U*-test after dividing the subjects according to age groups. The 2.5% and 97.5% percentiles were calculated and defined as the lower and upper limits of the normal range, respectively.

Study group

The results with respect to normal ranges of salivary S-IgA, IgA (2) in serum, and T-helper cells (27) were analyzed using the Binomial Test procedure, after conversion of the results into dichotomous variables (35) (values < lower normal limit vs. values ≥ lower normal limit). The probability parameter for values below the normal range was set at 0.1. To test the mutual dependence of serum IgA and T-helper lymphocytes on S-IgA, a partial correlation procedure was performed to calculate partial correlation coefficients, while controlling the effect of the sampling point (months after transplantation).

The results are presented at each sampling time as box plots, including minimums, 1st quartiles, medians, 3rd quartiles, maximums, extremes, and outliers. Differences between autologous and allogeneic recipients were examined using the Mann–Whitney *U*-test.

All analyses were performed with the SPSS, version 9.0.1. software (SPSS Inc., Chicago, IL).

Results

Control group

The mean S-IgA salivary concentration in the control group was 1654 ± 1836 mg/l (median = 868 mg/l), though the values were not normally distributed. There was a significant difference between subjects 7–14 years and those 14–25 years of age ($P = 0.02$).

The range of values between the 2.5th and 97.5th percentile in 7–14-year-old children was 143–6204 mg/l, vs. 243–8160 mg/l in 14–25-year-old subjects.

Study group

Recovery of immunoglobulin A in saliva and serum

The concentrations of salivary and serum IgA, which were within normal limits in two-thirds of the patients at the time of HSCT, evolved in the same direction thereafter, reaching a nadir followed by a return to normal levels. The S-IgA concentrations reached their nadir during months 3 and 4 after HSCT and, by month 5, their distribution (by binomial test) was similar to that observed in our reference population. Over two-thirds of the patients had normal S-IgA concentrations 1 year after transplantation (Fig. 1 and 2).

Although the serum IgA concentrations evolved in a similar direction (Fig. 3 and 4), the evolution was slower than that of S-IgA, reaching a nadir in the 6th month after HSCT. After 18 months following HSCT, the distribution of serum IgA concentrations (by binomial test) was similar to that found in a normal population.

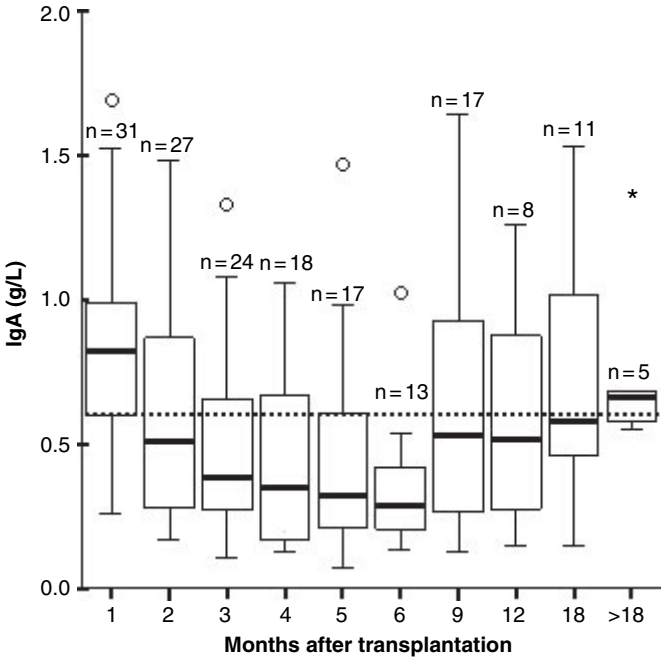

Fig. 3. Time course of IgA in serum after HSCT. Dotted line = lower level of normal range for mean of all patients (13.6 years); o = outliers; * = extremes.

Reconstitution of T-helper lymphocytes

The numbers of T-helper cells decreased rapidly to their lowest levels and remained significantly below normal for 18 months (Fig. 5 and 6), after which they returned to normal (by binomial test). Taking into consideration the timing of sampling after HSCT, we found a significant correlation between serum IgA concentrations and percentages of T-helper lymphocytes (partial correlation coefficient = 0.3793, $P < 0.001$). In contrast, there was no correlation between the concentrations of S-IgA and serum IgA (partial correlation coefficient = 0.1201, $P = 0.224$), or between the concentrations of S-IgA and percentages of T-helper lymphocytes (partial correlation coefficient = 0.1809, $P = 0.092$) when the effect of 'months after HSCT' was controlled for. The reconstitution of S-IgA, serum IgA, and T-helper cells was similar in autologous and allogeneic recipients.

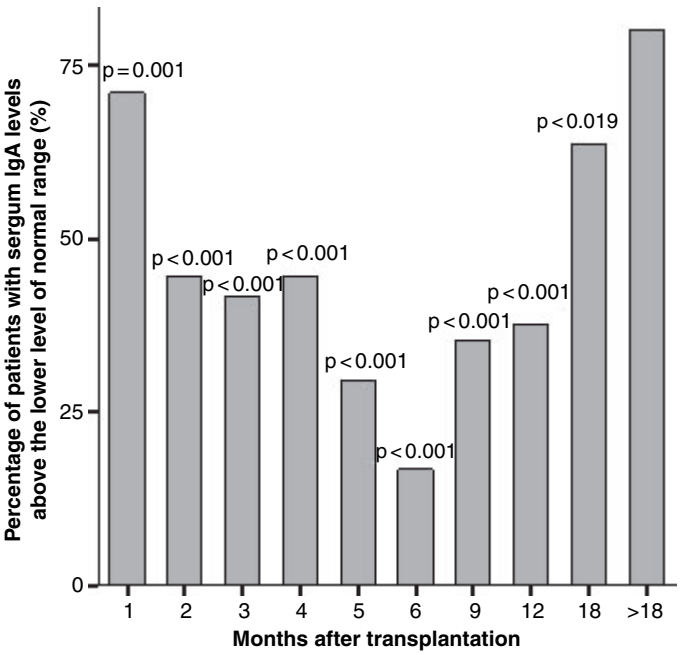

Fig. 4. Reconstitution of IgA in serum after HSCT with regard to normal range. Significance level for difference of distribution between normal population and patients is indicated (binomial test).

Discussion

Control group

Because of the inconsistent reports of, or missing reference values for, S-IgA in the saliva of children, we chose to measure S-IgA in a large group of young, healthy volunteers. The considerable scatter of values reported in the literature and measured in our study may be due to the influence of multiple factors, including age, variations in salivary flow rate, circadian and seasonal variations, environmental pollution, and stress, on the secretion of S-IgA (1, 4, 19, 24, 30, 32, 39, 42, 44). Several of these factors are confounding

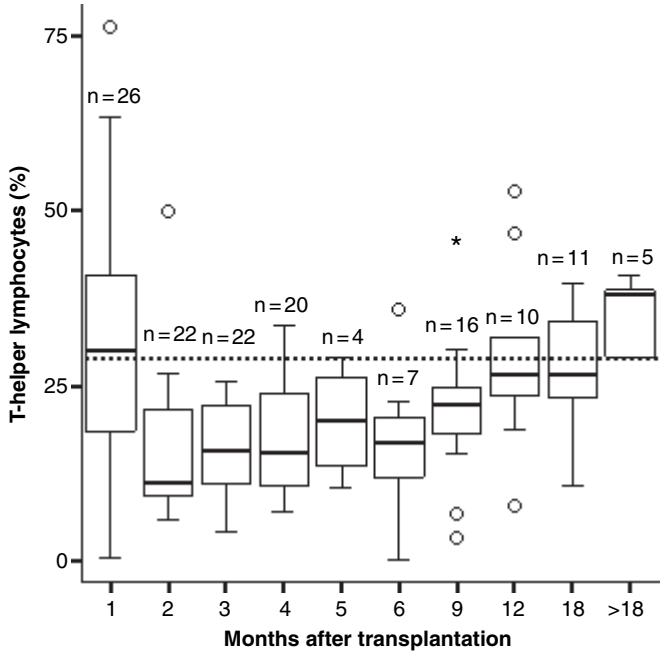

Fig. 5. Time course of T-helper lymphocytes after HSCT. Dotted line = lower level of normal range; o = outliers; * = extremes.

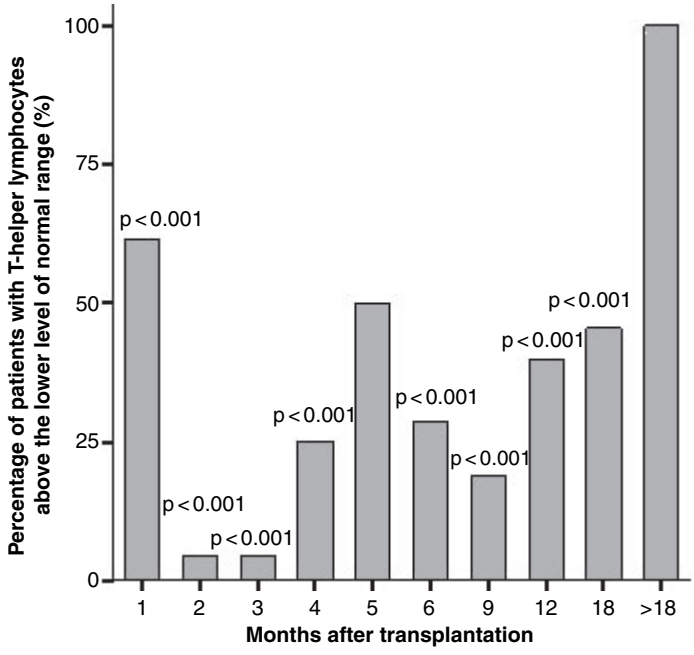

Fig. 6. Reconstitution of T-helper cells after HSCT with regard to normal range. Significance level for difference of distribution between normal population and patients is indicated (binomial test).

and difficult to control, a limitation of this and other studies. Furthermore, the measurements of S-IgA are influenced by the sampling method (1). These observations highlight the importance of defining accurately both the timing of sampling and the procedure of saliva collection. Because our S-IgA measurements in the control group did not fit a Gaussian distribution, we presented our data as a range of percentiles between 2.5 and 97.5. Therefore, comparisons between our results and those published by others may be problematic. On the other hand, few comparisons between patients who had undergone HSCT and healthy controls have been reported. Chaushu et al. (6) studied 30 patients between the ages of 11 and 60 years and nine healthy controls between the ages of 20 and 60 years. Norhagen et al. (41) studied 10 patients 7–28 years of age, and 20 adult controls whose ages were not specified. Other studies did not include healthy control groups (7, 8, 10). Consequently, comparative measurements of S-IgA concentrations were needed, particularly in children and adolescents. Unlike Chaushu et al. (6) we did not use a parotid-salivary-gland-cup to collect the saliva, but favored the Salivette^{TM} system because of the young age of our patients and the predictable development of oral mucous membrane lesions in the post-transplantation period.

Recovery of immune function

Our results pertaining to the recovery of S-IgA, serum IgA and T-helper cells after HSCT were generally similar to the observations made by others (5–9, 11, 20, 25, 26, 34, 40, 41, 45, 46). The rarely published (40) long-term evolution of these variables after HSCT within the same group allowed a precise definition of their time course. Though the serum IgA and S-IgA concentrations were similar, the S-IgA concentrations normalized earlier, reaching a nadir at 3–4 months after transplantation (vs. 6 months for serum IgA), and a clear recovery between 6 and 12 months (compared with >18 months for serum IgA). In previous studies, S-IgA reached a nadir within 6 months, and normalized up to 1 year after HSCT (6–9, 40, 41), whereas the lowest serum concentrations of IgA were measured after 3–5 months, and their recovery >12 months after HSCT (5, 11, 20, 25, 26, 34, 40, 45, 46). In a study comparing the course of salivary with that of serum immunoglobulins after HSCT, Norhagen et al. also observed a faster recovery of the salivary immunoglobulins (40).

Several studies, especially in mice, have suggested that the production of mucosal IgA is T-cell independent (16, 33, 36, 43). Epithelial and dendritic cells play an important role in the growth, differentiation, and transformation of B-cell isotype into S-IgA-secreting plasma cells (3, 10, 15, 16, 31, 33, 36, 43). We hypothesize that this explains the more rapid recovery of S-IgA than serum IgA after HSCT. This

is supported by the similar reconstitution patterns of serum IgA and T-helper cells, which both differed significantly from that of S-IgA, and by the correlation between the density of T-helper cells and serum IgA, but not S-IgA concentrations.

In conclusion, the recovery of S-IgA was faster than that of serum IgA. The reconstitution of S-IgA appears to be independent of the recovery of T-helper cell immunity in the peripheral blood.

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