JCI The Journal of Clinical Investigation

Immunopathophysiological aspects of an emerging neonatal infectious disease induced by a bacterial superantigen

Naoto Takahashi, … , Hiroshi Nishida, Takehiko Uchiyama

J Clin Invest. 2000;106(11):1409-1415. https://doi.org/10.1172/JCI10396.

Article

We recently discovered an emerging neonatal infectious disease, neonatal toxic shock syndrome–like (TSS-like) exanthematous disease (NTED), which is induced by a superantigen, TSS toxin-1 (TSST-1), produced by methicillin-resistant *Staphylococcus aureus* (MRSA). Here, we analyzed the activation and the response of TSST-1–reactive Vβ2$^+$ T cells in NTED patients during the acute and recovery phases and in asymptomatic infants exposed to MRSA. In the acute phase, Vβ2$^+$ T cells were anergic to stimulation with TSST-1 and underwent marked expansion, but by 2 months after disease onset, their numbers had declined to about 10% of the control level. Although the percentage of Vβ2$^+$ T cells in the ten asymptomatic neonatal MRSA carriers was within the control range, these individuals could be divided into two groups on the basis of Vβ2$^+$ T-cell activation. Vβ2$^+$CD4$^+$ T cells from three of these infants (Group 1) highly expressed CD45RO and were anergic to TSST-1, whereas in the other seven asymptomatic neonatal MRSA carriers (Group 2), these cells expressed CD45RO at the control level and were highly responsive to stimulation with TSST-1. The serum anti–TSST-1 IgG Ab titer was negligible in the four NTED patients in the acute phase and the three asymptomatic neonatal MRSA carriers in Group 1, but it was high in the seven asymptomatic carriers in Group 2. We suggest that maternally derived anti–TSST-1 IgGs […]

Immunopathophysiological aspects of an emerging neonatal infectious disease induced by a bacterial superantigen

Naoto Takahashi,[1] Hidehito Kato,[2] Ken'ichi Imanishi,[2] Keishi Miwa,[3] Sadao Yamanami,[4] Hiroshi Nishida,[1] and Takehiko Uchiyama[2]

[1]Maternal and Perinatal Center, and
[2]Department of Microbiology and Immunology, Tokyo Women's Medical University School of Medicine, Tokyo, Japan
[3]Medical Devices and Diagnostics Research Laboratories, Toray Industries Inc., Ohtsu, Japan
[4]Neonatal Center, Kawaguchi Municipal Medical Center, Kawaguchi, Japan

Address correspondence to: Naoto Takahashi, Maternal and Perinatal Center, Tokyo Women's Medical University School of Medicine, 8-1 Kawadacho, Shinjuku-ku, Tokyo, Japan 162-8666.
Phone: 81-3-3355-3010; Fax: 81-3-5269-7345; E-mail: naoto-t@xa2.so-net.ne.jp.

Received for publication May 22, 2000, and accepted in revised form October 23, 2000.

We recently discovered an emerging neonatal infectious disease, neonatal toxic shock syndrome–like (TSS-like) exanthematous disease (NTED), which is induced by a superantigen, TSS toxin-1 (TSST-1), produced by methicillin-resistant *Staphylococcus aureus* (MRSA). Here, we analyzed the activation and the response of TSST-1–reactive Vβ2$^+$ T cells in NTED patients during the acute and recovery phases and in asymptomatic infants exposed to MRSA. In the acute phase, Vβ2$^+$ T cells were anergic to stimulation with TSST-1 and underwent marked expansion, but by 2 months after disease onset, their numbers had declined to about 10% of the control level. Although the percentage of Vβ2$^+$ T cells in the ten asymptomatic neonatal MRSA carriers was within the control range, these individuals could be divided into two groups on the basis of Vβ2$^+$ T-cell activation. Vβ2$^+$CD4$^+$ T cells from three of these infants (Group 1) highly expressed CD45RO and were anergic to TSST-1, whereas in the other seven asymptomatic neonatal MRSA carriers (Group 2), these cells expressed CD45RO at the control level and were highly responsive to stimulation with TSST-1. The serum anti–TSST-1 IgG Ab titer was negligible in the four NTED patients in the acute phase and the three asymptomatic neonatal MRSA carriers in Group 1, but it was high in the seven asymptomatic carriers in Group 2. We suggest that maternally derived anti–TSST-1 IgGs helps to suppress T-cell activation by TSST-1 and protects infants from developing NTED.

J. Clin. Invest. **106**:1409–1415 (2000).

Introduction

Several years ago, we saw a number of neonates who developed systemic exanthema, fever, low-positive serum C-reactive protein (CRP) values, and thrombocytopenia within the first week of life and described this disorder as a new disease entity (1, 2). Subsequently, several research groups, including our own, found that virtually all of the neonatal patients with this disease had been colonized by methicillin-resistant *Staphylococcus aureus* (MRSA) that produced selectively toxic shock syndrome (TSS) toxin-1 (TSST-1) (3–6), which has superantigenic activity (7, 8). This finding suggested a close relationship between this disease and TSS, which is caused by overactivation of TSST-1–reactive T cells (8–11). We found recently that Vβ2$^+$ T cells, which are the major TSST-1–reactive human T cells (12), were polyclonally expanded in the neonatal patients, and we named this disease neonatal TSS-like exanthematous disease (NTED) (13). NTED regressed spontaneously without antibiotic therapy in full-term neonates, but most preterm neonates developed severe symptoms (13). According to the responses to our questionnaires, neonates fulfilling the clinical criteria of NTED were observed in 25.7% (19/74) of major neonatal care units in Japan in 1995 and in 70.8% (63/89) in 1998, indicating that the incidence of NTED in Japan has been increasing. This finding is thought to be related to the conversion of MRSA into the TSST-1–producing type in Japan (14). There is apprehension that NTED will become a widespread disease throughout the world, because MRSA has been spreading on a global level (15, 16).

NTED, like TSS, is caused by the overactivation of TSST-1–reactive T cells (13). There are several immunological points that must be resolved to obtain clues that will allow us a comprehensive understanding of NTED and will enable the development of methods of prevention. First, a biphasic response consisting of a transient massive expansion and a subsequent protracted anergic and deleted state has been found to be induced in superantigen-reactive T-cell populations in experiments in mice (7, 8, 17–19). It remains to be elucidated whether the same response pattern is induced in NTED patients. Second, around 20% of the neonates in the neonatal

Table 1
Clinical profiles of the NTED patients, asymptomatic neonatal MRSA carriers, and MRSA-free neonates

	GA (wk)	BW (kg)	Postnatal day Fever onset	Exanthema manifested	Maximum[A] WBC ($\times 10^3/\mu l$)	Lym	Minimum[A] Platelet ($\times 10^3/\mu l$)	Maximum CRP (mg/l)	MRSA culture Swabs Nasal	Oral	Umbil	Stools
P1	38	2.9	2	4–6	14.4	5.0	98	19	+	–	+	+
P2	33	1.3	–	3–5	18.4	8.8	127	28	nd	+	+	+
P3	37	2.3	2	3–4	12.9	8.3	86	43	+	–	+	+
P4	39	2.9	2	3–4	16.2	11.5	80	36	nd	+	+	nd
Asymptomatic MRSA carriers												
Ca1	39	3.1	–	–	12.3	4.0	444	<3.0	+	–	+	+
Ca2	39	3.2	–	–	8.8	3.7	nd	<3.0	–	–	+	–
Ca3	38	3.7	–	–	8.0	2.1	288	<3.0	+	–	+	nd
Ca4	38	3.0	–	–	6.7	2.0	456	<3.0	+	–	+	+
Ca5	38	3.0	–	–	8.4	5.2	234	<3.0	+	–	+	nd
Ca6	38	2.9	–	–	6.5	1.7	245	<3.0	–	–	+	+
Ca7	38	2.6	–	–	7.8	4.1	502	<3.0	–	–	+	–
Ca8	40	3.7	–	–	9.5	5.4	439	<3.0	–	–	+	+
Ca9	40	4.2	–	–	9.6	5.6	402	<3.0	+	–	+	nd
Ca10	38	3.0	–	–	8.4	5.2	234	<3.0	–	–	+	nd
MRSA-free neonates (mean ± SD)[B]												
	38.6 ±1.1	2.9 ±0.2	–	–	8.2 ±1.5	5.7 ±0.9	446 ±115	<3.0	–	–	–	–

[A]The white blood cell counts and platelet counts were maximal and minimal, respectively, on day 2 or 3 after the onset of exanthema in the four NTED patients and were examined on postnatal day 5 in the asymptomatic MRSA carriers and MRSA-free neonates. [B]Mean ± SD in the eight MRSA-free neonates on postnatal day 5. GA, gestational age; BW, birth weight; Lym, lymphocyte count; +, MRSA isolated; –, MRSA not isolated; nd, not done.

care unit of Tokyo Women's Medical University Hospital are MRSA carriers, and 10% of the 20% have manifested symptoms of NTED. We speculated that T-cell activation by TSST-1 was induced in a certain proportion of asymptomatic neonatal MRSA carriers and that a large proportion of them was protected from the development of NTED by the transplacental transfer of anti–TSST-1 Ab of maternal origin.

In the present study we obtained evidence indicating that long-lasting immunological tolerance was induced in the $V\beta2^+$ T cells of the NTED patients, that activation of $V\beta2^+$ T cells occurred in a certain proportion of asymptomatic neonatal MRSA carriers, and that anti–TSST-1 IgG Ab of maternal origin plays a protective role in preventing the development of NTED. We discuss the implications of our findings in relation to the pathogenetic mechanism underlying NTED and its prevention.

Methods

Neonates examined in the present study. Four NTED patients, ten asymptomatic neonatal MRSA carriers and eight MRSA-free neonates were registered as the subjects of this study. The NTED patients had been admitted to the neonatal care unit of Tokyo Women's Medical University Hospital or Kawaguchi Municipal Medical Center and had been diagnosed on the basis of the clinical criteria established by us for NTED, erythema plus at least one of the following three manifestations: thrombocytopenia, a low-positive serum CRP value, and fever (1, 2, 13). Nasal, oropharyngeal and umbilical swabs, and stools, collected from neonates on postnatal day 3, were examined for the presence of MRSA to select asymptomatic MRSA-carriers and MRSA-free neonates. After obtaining the informed consent of their parents, peripheral blood samples were collected from the NTED patients in the acute phase and in the recovery phase and from the asymptomatic MRSA carriers and MRSA-free neonates on postnatal day 5.

Reagents and mAb's. FITC-conjugated MPB2D5 (anti-Vβ2), CH92 (anti-Vβ3), SFCI12T4D11 (anti-CD4), phycoerythrin-cyanin 5.1–conjugated (PC5-conjugated) UCHT1 (anti-CD3), 13B8.2 (anti-CD4), and B9.11 (anti-CD8) were purchased from Coulter Corp. (Hialeah, Florida, USA). Phycoerythrin-conjugated (PE-conjugated) UCHL1 (anti-CD45RO) and SK1 (anti-CD8) were purchased from Becton Dickinson Immunocytometry Systems (Mountain View, California, USA). TSST-1 and staphylococcal enterotoxin A (SEA) were purchased from Toxin Technology (Sarasota, Florida, USA). The RPMI-1640 culture medium used contained 10% FCS and 5×10^{-5} M 2-ME. The recombinant IL-2 used in the IL-2 assay was kindly provided by Takeda Chemical Industries (Kyoto, Japan).

Characterization of the immunological phenotypes of T cells by flow cytometry. PBMCs were isolated by Ficoll-Conray density-gradient centrifugation, as described previously (20, 21). To examine the percentage of $V\beta2^+$ T cells (reactive with TSST-1) or $V\beta3^+$ T cells (reactive with staphylococcal enterotoxin B; SEB) (7, 8), PBMCs were stained with several combinations of adequate PC5-, FITC-, or PE-conjugated mAb's and examined by three-color flow-cytometry analysis using a EPICS XL flow cytometer (Coulter Corp.), as described previously (20, 21).

Assay of superantigen-induced IL-2 production by mononuclear cells. Isolated PBMCs (2×10^5) were stimulated with 10 ng of TSST-1 or SEA per milliliter in 200-μl vol-

umes in round-bottom 96-well culture plates (Becton Dickinson, Franklin Lakes, New Jersey, USA) for various durations. IL-2 activity in the culture supernatants was determined by using IL-2–dependent CTLL-2 cells, as reported previously (20, 21). Data are shown as units of IL-2 per milliliter.

Measurement of anti–TSST-1 Ab's by ELISA. Titers of anti–TSST-1 Ab's (IgG and IgM) were measured by ELISA, as described previously (22). Briefly, serum diluted to 1:1000 and 1:100 for titration of IgG and IgM anti–TSST-1 Ab's, respectively, was applied to either TSST-1–precoated or noncoated plates, in duplicate. Peroxidase-conjugated rabbit anti-human IgG or IgM Ab's (Organo Teknika Corp., West Chester, Pennsylvania, USA) and tetramethyl benzidine (Sigma Chemical Co., St. Louis, Missouri, USA) were added to the plates, and the Ab titers were determined based on the OD at 450 nm. Data are shown as OD.

Statistical analysis. Statistical analysis was performed using the Mann-Whitney's *U*-test. *P* values less than 0.05 were considered significant.

Results

Clinical profiles and laboratory data of NTED patients, asymptomatic MRSA carriers, and MRSA-free neonates. The clinical profiles of the four NTED patients, ten asymptomatic MRSA carriers, and eight MRSA-free neonates examined are shown in Table 1. NTED patient P2 was admitted to the intensive care unit soon after birth due to being a preterm infant. The other NTED patients P1, P3, and P4, were transferred from the newborn nursery to the intensive care unit after the onset of NTED. Systemic exanthema, fever, low-positive serum CRP values, and thrombocytopenia were consistently observed in the full-term neonates, P1, P3, and P4. No fever was noted in the preterm neonate, P2, who exhibited apnea attacks and food intolerance and had symptomatic patent ductus arteriosus. While all three full-term neonates with NTED recovered spontaneously without any antibiotic therapy, the preterm neonate, P2, recovered after treatment with vancomycin. On day 2–3 after the onset of the exanthema, the white blood cell (WBC) and lymphocyte counts of all four NTED patients were high, and their platelet counts were low. With the exception of a slight increase in WBC count in one MRSA carrier, Ca1, the laboratory data in the ten asymptomatic neonatal MRSA carriers were almost the same as in the MRSA-free neonates. It is noteworthy that the umbilicus was a major site of colonization by TSST-1–producing MRSA. All asymptomatic MRSA carriers and MRSA-free neonates remained in the newborn nursery and were discharged from the hospital without any clinical manifestations. None of the ten asymptomatic MRSA carriers showed any symptoms during the first month of life.

Selective expansion and specific anergy induction in Vβ2⁺ T cells in acute-phase NTED patients. The immunological state of the four NTED patients was investigated. First, the peripheral blood mononuclear (PBM) T cells of the NTED patients and MRSA-free neonates were examined to determine the percentage of Vβ2⁺ T cells. The results are summarized in Table 2. The number of CD3⁺ T cells was increased in three of the four NTED patients, and the percentages of Vβ2⁺ CD4⁺ and Vβ2⁺ CD8⁺ T cells were significantly higher in the four NTED patients than in the eight MRSA-free neonates. A high percentage of these expanded Vβ2⁺ T cells in the NTED patients expressed CD45RO, whereas only a low or negligible percentage of the Vβ2⁺ T cells of the eight MRSA-free neonates expressed CD45RO. The percentages of Vβ3⁺CD4⁺ and Vβ3⁺CD8⁺ T cells of the NTED patients that are reactive with staphylococcal enterotoxin B (SEB) (7, 8) were low (Table 2), as reported previously (13). These results indicate that Vβ2⁺ T cells

Table 2
Expansion and anergy induction to TSST-1 in TCR Vβ2⁺ T cells from NTED patients in the acute phase

				Percentage of					IL-2 production (U/ml)[C]		
				Vβ2+ T cells (CD45RO⁺)[B]		Vβ3+ T cells (CD45RO⁺)			Period of stimulation		
	Age (days)	T cells (×10³/μl)	CD4/CD8 ratio	CD4⁺	CD8⁺	CD4⁺	CD8⁺	Toxin	8 h	24 h	48 h
NTED patients											
P1	5	5.5	2.9	27.2[A] (86.0)[B]	36.7 (86.2)	5.7 (5.7)	7.4 (4.8)	TSST-1	<0.1	<0.1	<0.1
								SEA	1.0	5.3	16.2
P2	4	6.4	5.0	29.5 (96.7)	29.0 (69.7)	6.8 (5.7)	8.5 (2.8)	TSST-1	<0.1	<0.1	<0.1
								SEA	0.3	13.0	28.0
P3	4	4.3	4.6	25.4 (85.1)	26.4 (85.6)	nd	nd	TSST-1	1.7	0.6	<0.1
								SEA	1.8	9.5	20.0
P4	5	9.8	6.0	25.7 (57.1)	21.7 (44.2)	nd	nd	TSST-1	<0.1	0.8	2.0
								SEA	1.5	14.0	63.0
MRSA-free neonates (controls)[D]											
	5	4.4 ± 1.7	4.0 ± 2.0	11.5 ± 1.5 (5.1 ± 2.5)	6.1 ± 0.9 (1.0 ± 1.1)	5.3 ± 2.7 (6.2 ± 1.8)	4.9 ± 2.7 (0.8 ± 0.8)	TSST-1	2.7 ± 0.9	33.6 ± 5.2	76.3 ± 13.4
								SEA	2.8 ± 0.6	40.1 ± 8.8	86.0 ± 55.4

[A]Percentage of Vβ2⁺CD4⁺ and Vβ2⁺CD8⁺ T cells or Vβ3⁺CD4⁺ and Vβ3⁺CD8⁺ T cells among PBM T cells. [B]Percentage of the CD45RO⁺ fraction among the Vβ2⁺CD4⁺ and Vβ2⁺CD8⁺ T cells or Vβ3⁺CD4⁺ and Vβ3⁺CD8⁺ T cells. [C]PBMCs (2×10^5/culture) were stimulated in vitro with 10 ng of TSST-1 or SEA per milliliter for indicated periods, and the culture supernatants were assayed for IL-2 activity. [D]Mean ± SD in the eight MRSA-free neonates on postnatal day 5. nd, not done.

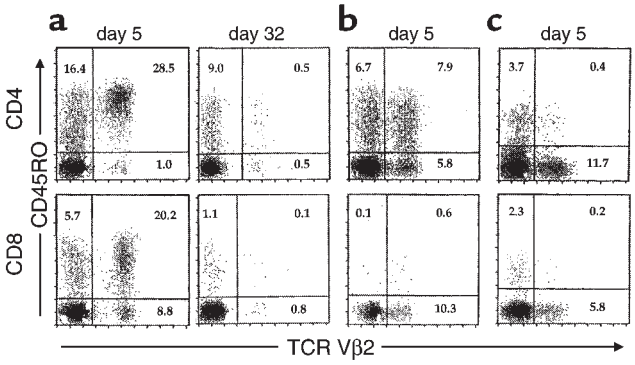

Figure 1
Expression of T-cell receptor Vβ2 versus CD45RO on CD4+ and CD8+ T cells obtained from an NTED patient in the acute and recovery phases, an asymptomatic MRSA carrier, and an MRSA-free neonate. The percentage of Vβ2+ T cells and the expression levels of CD45RO by Vβ2+CD4+ and Vβ2+CD8+ T cells were examined in the preterm NTED patient P2 on postnatal days 4 and 32 (**a**), in the asymptomatic neonatal MRSA carrier Ca1 on postnatal day 5 (**b**), and in an MRSA-free neonate on postnatal day 5 (**c**). The numbers are the percentages of stained cells in each area.

exhibited selective activation and expansion induced by TSST-1 in the NTED patients, irrespective of the CD4+ or CD8+ T-cell subsets. The flow-cytometric findings for the expansion of Vβ2+ T cells expressing CD45RO in patient P2 are shown in Figure 1a.

Second, to investigate whether the expanded TSST-1–reactive T cells retained their ability to respond to stimulation with TSST-1 in the NTED patients, we examined IL-2 production by PBM T cells from the NTED patients and MRSA-free neonates in response to in vitro stimulation with TSST-1 or an unrelated superantigen, SEA. As shown in Table 2, PBM T cells from MRSA-free neonates exhibited marked IL-2 production in response to stimulation with either TSST-1 or SEA. By contrast, the PBM T cells from the NTED patients exhibited no or only a minimum level of IL-2 production in response to stimulation with TSST-1, but exhibited IL-2 production that was substantial, although slightly lower than in the MRSA-free neonates, in response to stimulation with SEA. The results indicate that the expanded TSST-1–reactive Vβ2+ T cells of the NTED patients were specifically anergic to TSST-1, as seen in the superantigen-reactive T cells of mice injected with these superantigens (17–19).

Protracted deletion of Vβ2+ T cells in NTED patients. We monitored the Vβ2+ T cells in the peripheral blood of the four NTED patients for certain periods after the onset of the disease to determine their fate. The results are shown in Figure 2. The percentages of Vβ2+CD4+ T cells and Vβ2+CD8+ T cells in the four patients were quite high in the acute phase, as shown above, but decreased to around the levels of the MRSA-free neonates on postnatal day 5 (the control) within 10 days and to very low levels, around 10% of the control level, by 1 or 2 months after the onset of disease (Figure 2, a and b). The 5-month follow-up examination of patient P1 revealed 50% recovery of the deleted Vβ2+ T cells to the control level. The percentages of Vβ2+CD4+ and Vβ2+CD8+ T cells were almost the same in the eight MRSA-free neonates on postnatal day 5, the one MRSA-free neonate on postnatal day 39, and the seven healthy adults, respectively (Figure 2, a and b), indicating that the levels of Vβ2+CD4+ and Vβ2+CD8+ T cells do not change much with age in healthy individuals. These findings indicate that a biphasic response consisting of transient expansion and subsequent specific deletion was induced in the Vβ2+ T cells of the NTED patients.

Immunological state of TSST-1–reactive T cells in asymptomatic neonatal MRSA carriers. The immunological state of the asymptomatic neonatal MRSA carriers was investigated. First, PBM T cells from the ten asymptomatic neonatal MRSA carriers were examined for the expression of Vβ2 and CD45RO on postnatal day 5. Although the percentage of their Vβ2+ T cells was within the normal range for both CD4+ and CD8+ T cells, we found increased expression of CD45RO by Vβ2+CD4+ T cells in three (Ca1–Ca3) of the ten neonatal carriers (Table 3), suggesting that Vβ2+CD4+ T cells were acti-

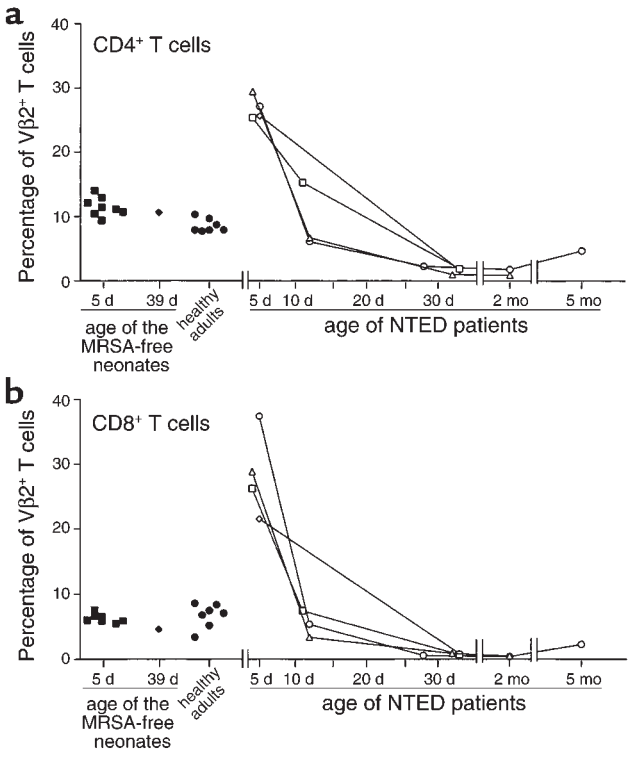

Figure 2
Fate of TCR Vβ2+ T cells in NTED patients. The four NTED patients in the acute and recovery phases, nine MRSA-free neonates on postnatal day 5 or 39, and seven healthy adults were examined for the percentage of Vβ2+CD4+ T cells (**a**) and Vβ2+CD8+ T cells (**b**) among PBMCs. NTED patients P1 (open circles), P2 (open triangles), P3 (open squares), and P4 (open diamonds) refer to the same cases as in Tables 1 and 2. Filled squares, MRSA-free neonates on postnatal day 5; filled diamonds, MRSA-free neonates on postnatal day 39; filled circles, healthy adults.

Table 3
Immunologic state of the asymptomatic neonatal MRSA carriers

MRSA carriers	T cells ($\times 10^3/\mu l$)	CD4 to CD8 ratio	Percentage of Vβ2+ T cells[A] (CD45RO+ fraction)[B] CD4+	CD8+	IL-2 production (U/ml)[C] Period of stimulation toxin	8 h	24 h
Group 1							
Ca1	2.9	2.2	13.7[A] (57.6)[B]	10.9 (5.1)	TSST-1	0.6	1.6
					SEA	1.4	32.0
Ca2	2.6	3	11.1 (55.8)	9.1 (5.7)	TSST-1	0.7	2.0
					SEA	1.0	23.0
Ca3	0.9	3.2	15.0 (27.5)	6.8 (4.4)	TSST-1	1.4	7.0
					SEA	2.6	15.0
Group 2							
Ca4	1.6	6.7	9.9 (10.2)	5.5 (0.1)	TSST-1	0.9	12.0
					SEA	1.0	25.0
Ca5	3.9	3.9	14.7 (4.1)	6.8 (4.3)	TSST-1	3.6	20.0
					SEA	4.5	16.2
Ca6	1.3	3.6	13.7 (6.1)	7.6 (0.3)	TSST-1	1.1	23.0
					SEA	0.6	25.0
Ca7	3.2	5.9	7.5 (3.9)	6.7 (0.0)	TSST-1	nd	nd
					SEA	nd	nd
Ca8	4.1	5.4	10.5 (3.2)	6.6 (0.9)	TSST-1	nd	nd
					SEA	nd	nd
Ca9	4.3	1.8	9.4 (4.6)	5.9 (0.0)	TSST-1	nd	nd
					SEA	nd	nd
Ca10	3.3	2.9	10.7 (1.9)	5.9 (1.8)	TSST-1	nd	nd
					SEA	nd	nd
MRSA-free neonates[D]							
	4.4 ± 1.7	4.0 ± 2.0	11.5 ± 1.5	6.1 ± 0.9	TSST-1	2.7 ± 0.9	33.6 ± 5.2
			(5.1 ± 2.5)	(1.0 ± 1.1)	SEA	2.8 ± 0.6	40.1 ± 8.8

Ten asymptomatic neonatal MRSA carriers were examined on postnatal day 5 for expression of CD45RO by Vβ2+ PBM T cells and TSST-1–induced IL-2 production by PBMCs. They were divided arbitrarily into two groups on the basis of activation level of Vβ2+CD4+ T cells in three cases (Ca1–Ca3) with high CD45RO expression by Vβ2+CD4+ T cells and low TSST-1–induced IL-2 production by PBMCs (Group 1) and seven cases (Ca4–Ca10) with low CD45RO expression by Vβ2+CD4+ T cells and high IL-2 production (Group 2). Although TSST-1–induced IL-2 production was not examined in four neonates (Ca7–Ca10), we classified them into Group 2 on the basis of their low expression of CD45RO by Vβ2+ CD4+ T cells. [A]Percentage of Vβ2+ T cells among PBM CD4+ or CD8+ T cells. [B]Percentage of CD45RO+ cells among Vβ2+CD4+ and Vβ2+CD8+ T cells ($P = 0.017$ between Groups 1 and 2). [C]PBMCs (2×10^5/culture) were stimulated in vitro with 10 ng of TSST-1 or SEA per milliliter for 8 hours or 24 hours, and the culture supernatants were assayed for IL-2 activity. [D]Mean ± SD in eight MRSA-free neonates on postnatal day 5. nd, not done.

vated by TSST-1 in these neonates and intact in the other seven neonates. The flow-cytometric findings in Ca1 are shown in Figure 1b.

We then examined IL-2 production by PBM T cells in response to in vitro stimulation with TSST-1 and SEA in the above three (Ca1–Ca3) of the ten asymptomatic neonatal MRSA carriers and in three (Ca4–Ca6) of the other seven carriers. The PBM T cells of neonates Ca1–Ca3 did not produce IL-2 in response to stimulation with TSST-1 but responded normally to stimulation with SEA. By contrast, the PBM T cells from neonates Ca4-Ca6 responded normally to both TSST-1 and SEA (Table 3). The IL-2 response of neonates Ca7–Ca10 was not examined. The results indicated that the Vβ2+CD4+ T cells of neonates Ca1–Ca3 were anergic to TSST-1, but that the Vβ2+CD4+ T cells of neonates Ca4–Ca6 were intact. The PBM T cells of neonates Ca7–Ca10 presumably responded normally to both TSST-1, and SEA because the level of expression of CD45RO by their Vβ2+CD4+ T cells was within the control range (Table 3).

The results indicated that the Vβ2+CD4+ T cells of three (Ca1–Ca3) of the ten asymptomatic neonatal MRSA carriers had been activated by TSST-1 and suggested that they were not activated by TSST-1 in the other seven, but were intact. We therefore arbitrarily divided the ten asymptomatic neonatal MRSA carriers into two groups on the basis of Vβ2+ T-cell activation: Group 1, neonatal carriers (Ca1–Ca3) with Vβ2+CD4+ T cells activated by TSST-1; and Group 2, carriers (Ca4–Ca10) with intact Vβ2+CD4+ T cells.

Protective role of anti–TSST-1 Ab of maternal origin against the influence of TSST-1. Neonates are generally protected against various infectious agents by specific IgG Ab's transferred transplacentally from their mothers (23). As anti–TSST-1 Ab's are known to play a protective role against the development of TSS in adults (24), it seems likely that anti–TSST-1 Ab's transferred placentally from their mothers play a role in protecting neonates from developing NTED. We determined the titers of anti–TSST-1 IgG and IgM Ab's in the serum of the NTED patients in the acute and recovery phases, the asymptomatic neonatal MRSA carriers, and the MRSA-free neonates on postnatal day 5 (Figure 3). In the four neonates with NTED, the serum anti–TSST-1 IgG Ab titer was negligible in the acute phase of the disease, but

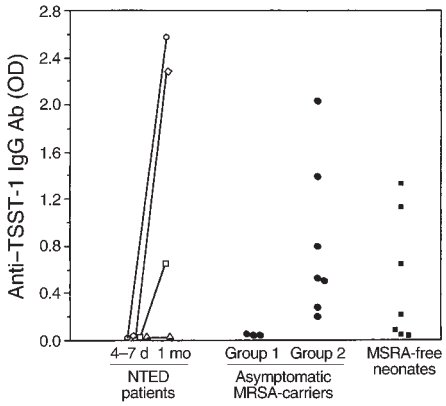

Figure 3
Titers of anti–TSST-1 IgG Ab in NTED patients, asymptomatic MRSA carriers, and MRSA-free neonates. Anti–TSST-1 IgG Ab titers were examined by ELISA in the serum of the four NTED patients P1 (open circles), P2 (open triangles), P3 (open squares), P4 (open diamonds) in the acute and recovery phases, ten asymptomatic neonatal MRSA carriers on postnatal day 5 (filled circles), and seven MRSA-free neonates on postnatal day 5 (filled squares). They were determined based on the OD at 450 nm.

increased within 1 month in the patients P1, P3, and P4, but not in the preterm patient P2. In the asymptomatic neonatal MRSA carriers, the anti–TSST-1 IgG Ab titer was almost negligible in the three Group 1 neonates with $V\beta2^+CD4^+$ T cells activated by TSST-1 (Table 3), but was high (0.2 or more) in the Group 2 neonates without $V\beta2^+CD4^+$ T cells activated by TSST-1. The anti–TSST-1 Ab titer was low (less than 0.2) in three of the eight MRSA-free neonates examined and high (more than 0.2) in the other five. The anti–TSST-1 IgM Ab titer was negligible soon after their birth (4–7 days) in all four NTED patients, the asymptomatic neonatal MRSA carriers, and the MRSA-free neonates (data not shown). At 1 month of life, the anti–TSST-1 IgM Ab titer had increased only slightly, from less than 0.01 to 0.02–0.03, in three NTED patients.

Discussion

In the present study, we examined several immunological aspects of a newly discovered neonatal disease, NTED, induced by superantigen TSST-1 in order to obtain clues that could lead to a comprehensive understanding of the pathogenetic mechanism underlying NTED and the development of methods to prevent the disease. The results clarified the immunological events that occurred in TSST-1–reactive human T cells and the protective role of anti–TSST-1 IgG Ab's transferred transplacentally against the development of NTED.

The TSST-1–reactive $V\beta2^+$ T cells that exhibited expansion in the acute phase of NTED were found to be anergic to TSST-1 (Table 2), and the $V\beta2^+$ T cells in the expanded state were subsequently eliminated to around 10% of the control level by 1–2 months after the onset of disease (Figure 2), indicating that a response pattern similar to that in mice injected with bacterial superantigens (7, 8, 17–19) was also induced in human neonates exposed to TSST-1. Recovery of the deleted $V\beta2^+$ T cells to 50% of the control level occurred at around 5 months (Figure 2), suggesting that 6 months or more are required for the recovery of deleted clones to normal levels in neonates. As in the NTED patients, expansion of $V\beta2^+$ T cells was found previously in adult TSS patients (ref. 11 and our unpublished data). The deletion of the expanded $V\beta2^+$ T cells, however, was not as profound or rapid in the adult TSS patients; the $V\beta/C\alpha$ ratio of the $V\beta2^+$ examined by the PCR method in two blood samples drawn 25 and 50 days after the onset of symptoms was still higher than that in the control (11). We think that the implications of the results of these studies in TSS in adults and NTED are important in terms of T-cell maturity in the neonatal period and the outcome of NTED.

According to Burnet's clonal selection theory (25), elimination of self-reactive lymphocytes occurs early in life, and the results of many experiments, including those of Billingham et al. (26), mainly using mice, have supported his concept and given rise to the notion that T cells in the neonatal period are intrinsically susceptible to anergy induction. This view has been refuted by reports suggesting that the T cells of neonatal and mature individuals are not qualitatively different, as reviewed by Stockinger (27). Recently, however, we observed that human $CD1a^-$ $CD4^+$ T cells in the final stage of maturation in the thymus and cord-blood $CD4^+$ T cells that supposedly had migrated recently from the thymus were susceptible to anergy induction by in vitro stimulation with TSST-1, whereas adult PBM $CD4^+$ T cells were resistant (20, 21), indicating the immaturity of human T cells during the neonatal period. On the basis of the results of the studies described above, we think that the rapid elimination of TSST-1–reactive $V\beta2^+$ T cells after their transient expansion in NTED patients is a reflection of the intrinsically immature state of the T cells resident in neonates. Because NTED is caused by overactivation of TSST-1–reactive T cells, mainly $V\beta2^+$ T cells (13), the rapid recovery from the illness without any complications in most full-term NTED patients (1, 2, 13) seems to be largely attributable to this immaturity of the T cells, the high susceptibility to anergy induction, and rapid deletion of $V\beta2^+$ T cells in the early neonatal period. The multiorgan failure seen in mature TSS patients could be caused by the persistent activated state of the $V\beta2^+$ T cells.

The percentages of $V\beta2^+CD4^+$ T cells and $V\beta2^+CD8^+$ T cells in the ten asymptomatic neonatal MRSA carriers examined were within the normal range (Table 3), however, they could be divided into two groups, Group 1 and Group 2, on the basis of the TSST-1–induced activation of their $V\beta2^+$ T cells (Table 3). The $V\beta2^+$ T cells in Group 1 neonates were activated by TSST-1, but they were intact and not activated in Group 2 neonates. These results suggest that the TSST-1–reactive T cells are activated by TSST-1 in

about 30% of neonatal MRSA-carriers, although the number of neonates examined was too low to evaluate the these findings statistically.

A question arises as to what factors divided the neonatal MRSA carriers into NTED patients and the Group 1 and Group 2 asymptomatic neonatal MRSA carriers. The view that NTED is caused by overactivation of TSST-1–reactive T cells (13) suggests that the level of activation of Vβ2$^+$ T cells governs the development of NTED. We think that the level of activation of Vβ2$^+$ T cells can be determined mainly by the amount of TSST-1 absorbed and anti–TSST-1 IgG Ab that can neutralize the superantigenic activity of TSST-1 in neonates. The level of Vβ2$^+$ T-cell activation in the four NTED patients was clearly higher than in the three Group 1 asymptomatic neonatal MRSA carriers, as shown in Table 2, Table 3, and Figure 1. Another clue as to the answer to the question was obtained from the analysis of the anti–TSST-1 IgG Ab titer in the NTED patients and the asymptomatic neonatal MRSA carriers. The results showed that the serum anti–TSST-1 IgG Ab titer was negligible in all four NTED patients and Group 1 neonates, but high in the Group 2 neonates in the early days after birth (Figure 3). We presume that higher amounts of TSST-1 were absorbed in the NTED patients than in the Group 1 neonates.

Anti–TSST-1 IgG Ab was found to be effective in blocking the superantigenic activity of TSST-1 and protecting against the development of NTED, as shown in Figure 3 and discussed above. This finding indicates that transfer of high amounts of anti–TSST-1 IgG Ab from mothers to their children through the placenta are effective in protecting against the development of NTED. The effectiveness of vaccination in preventing abnormal superantigen-induced reactions by a superantigen that has lost its superantigenic activity has been studied in animal experiments (28). Vaccination of pregnant women with attenuated TSST-1 may be one means of preventing the development of NTED in their children.

There are several questions as to whether TSST-1–reactive T cells of NTED patients retain long-term memory against TSST-1 that can be seen in the anergy induction to stimulation with TSST-1 and whether the TSST-1–reactive T cells in an activated state in the Group 1 neonates normalize with time. Does complete deletion of Vβ2$^+$ T cells occur in the recovery phase in NTED patients, when the patients are exposed to a high amounts of TSST-1? These questions are left to be clarified in future studies.

Acknowledgments

This work was supported by grants from the Ministry of Education, Science, Sports and Culture of Japan, the Ministry of Health and Welfare of Japan, and The Mother and Child Health Foundation. The authors thank M. Maruyama for identification of the MRSA carriers and I. Sakuma for collecting the blood specimens.

1. Takahashi, N., et al. 1995. A new exanthematous disease in newborn infants. *Acta Neonatologica Japonica.* **31**:371–377.
2. Takahashi, N., and Nishida, H. 1997. A new exanthematous disease with thrombocytopenia in newborn infants. *Arch. Dis. Child.* **77**:F79.
3. Makimoto, A., Kubo, M., and Kawakami, K. 1996. Neonatal thrombocytopenia with skin eruption, probably caused by exotoxin-producing methicillin-resistant *Staphylococcus aureus* (MRSA): clinical evaluation in 14 cases. *Journal of the Japan Pediatric Society.* **100**:609–615.
4. Matsuo, Y., et al. 1997. Clinical studies of early neonatal exanthematous disease with thrombocytopenia: in relation to methicillin resistant Staphylococcus aureus (MRSA). *J. Jpn. Soc. for Premature Newborn Med.* **9**:86–93.
5. Okada, T., Furukawa, S., Satomura, S., Hayabuchi, Y., and Ohta, A. 1997. Bacterial examinations of a new exanthematous disease in newborn infants. *Journal of the Japan Pediatric Society.* **101**:631–637.
6. Sakata, Y., et al. 1997. Exanthema of the newborn related to toxic shock syndrome toxin-1 (TSST-1). *Japanese Journal of Obstetrical, Gynecological and Neonatal Hematology.* **7**:146–150.
7. Kotzin, B.L., Leung, D.Y., Kappler, J., and Marrack, P. 1993. Superantigens and their potential role in human disease. *Adv. Immunol.* **54**:99–166.
8. Uchiyama, T., Yan, X.-J., Imanishi, K., and Yagi, J. 1994. Bacterial superantigens: mechanism of T cell activation by the superantigens and their role in the pathogenesis of infectious diseases. *Microbiol. Immunol.* **38**:245–256.
9. Uchiyama, T., et al. 1987. Study of the biological activities of toxic shock syndrome toxin-1: induction of the proliferative response and interleukin 2 production by T cells from human peripheral mononuclear cells stimulated with the toxin. *Clin. Exp. Immunol.* **68**:638–647.
10. Uchiyama, T., et al. 1989. Activation of human T cells by toxic shock syndrome toxin-1: the toxin binding structures expressed on human lymphoid cells acting as accessory cells are HLA class II molecules. *Eur. J. Immunol.* **17**:1803–1809.
11. Choi, Y., et al. 1990. Selective expansion of T cells expressing Vβ2 in toxic shock syndrome. *J. Exp. Med.* **172**:981–984.
12. Choi, Y., et al. 1989. Interaction of Staphylococcus aureus toxin "superantigens" with human T cells. *Proc. Natl. Acad. Sci. USA.* **86**:8941–8945.
13. Takahashi, N., et al. 1998. Exanthematous disease induced by toxic shock syndrome toxin 1 in the early neonatal period. *Lancet.* **351**:1614–1619.
14. Cui, L., Hanaki, H., and Hiramatsu, K. 1998. The epidemiology of MRSA producing TSST-1 and the influence of serum on the production of TSST-1 [in Japanese]. *Japanese Journal of Bacteriology.* **53**:155. (Abstr.)
15. Ayliffe, G.A. 1997. The progressive intercontinental spread of methicillin-resistant Staphylococcus aureus. *Clin. Infect. Dis.* **24**(Suppl. 1):S74–S79.
16. Archer, G.L. 1998. *Staphylococcus aureus*: a well-armed pathogen. *Clin. Infect. Dis.* **26**:1179–1181.
17. Kawabe, Y., and Ochi, A. 1990. Selective anergy of Vβ8$^+$, CD4$^+$ T cells in Staphylococcus enterotoxin B-primed mice. *J. Exp. Med.* **172**:1065–1070.
18. Rellahan, B.L., Jones, L.A., Kruisbeek, A.M., Fry, A.M., and Matis, L.A. 1990. In vivo induction of anergy in peripheral Vβ8+ T cells by staphylococcal enterotoxin B. *J. Exp. Med.* **172**:1091–1100.
19. Kuroda, K., et al. 1996. Implantation of IL-2-containing osmotic pump prolongs the survival of superantigen-reactive T cells expanded in mice injected with bacterial superantigen. *J. Immunol.* **157**:1422–1431.
20. Takahashi, N., Imanishi, K., Nishida, H., and Uchiyama, T. 1995. Evidence for immunologic immaturity of cord blood T cells. Cord blood T cells are susceptible to tolerance induction to in vitro stimulation with a superantigen. *J. Immunol.* **155**:5213–5219.
21. Imanishi, K., et al. 1998. Post-thymic maturation of migrating thymic single positive T cells: thymic CD1a$^-$ CD4$^+$ T cells are more susceptible to anergy induction by toxic shock syndrome toxin-1 than cord blood CD4$^+$ T cells. *J. Immunol.* **160**:112–119.
22. Terai, M., et al. 1995. The absence of evidence of staphylococcal toxin involvement in the pathogenesis of Kawasaki disease. *J. Infect. Dis.* **172**:558–561.
23. Wilson, C.B. 1990. Developmental immunology and role of host defenses in neonatal susceptibility. In *Infectious diseases of the fetus and newborn infant.* 3rd edition. J.S. Remington and J.O. Klein, editors. W.B. Saunders Co. Philadelphia, Pennsylvania, USA. 17–67.
24. Vergerout, J., et al. 1983. Prevalence of serum antibody to staphylococcal enterotoxin F among Wisconsin residents: implications for toxic shock syndrome. *J. Infect. Dis.* **148**:692–698.
25. Burnet, F.M. 1959. *The clonal selection theory of acquired immunity.* Cambridge University Press. Cambridge, United Kingdom. 208 pp.
26. Billingham, R.E., Brent, L., and Medawar, P.B. 1953. 'Actively acquired tolerance' of foreign cells. *Nature.* **172**:603–606.
27. Stockinger, B. 1996. Neonatal tolerance mysteries solved. *Immunol. Today.* **17**:249.
28. Bavari, S., Dyas, B., and Ulrich, R. 1996. Superantigen vaccines: a comparative study of genetically attenuated receptor-binding mutants of staphylococcal enterotoxin A. *J. Infect. Dis.* **174**:338–345.