

ORIGINAL ARTICLE

The association between DRESS and the diminished numbers of peripheral B lymphocytes and natural killer cells

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To cite this article: Yazicioglu M, Elmas R, Turgut B, Genchallac T. The association between DRESS and the diminished numbers of peripheral B lymphocytes and natural killer cells. *Pediatr Allergy Immunol* 2012; doi: 10.1111/j.1399-3038.2012.01268.x

Keywords

antibiotics; B cell; children; drug-induced hypersensitivity syndrome; drug reaction with eosinophilia and systemic symptoms; natural killer cell

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Accepted for publication 17 December 2011

DOI:10.1111/j.1399-3038.2012.01268.x

Abstract

Drug reaction with eosinophilia and systemic symptoms (DRESS) is a drug-induced, severe multiorgan system reaction whose exact pathogenesis remains unknown. This study aimed at evaluating specific changes in peripheral blood lymphocyte subtypes associated with DRESS during antibiotic treatment. We analyzed six patients with DRESS. A complete blood count and peripheral blood lymphocytes immunophenotyping were carried out at symptom onset and at follow-up visits. Acute-phase reactants and liver enzymes were measured in all patients. Other tests – viral serology, serum immunoglobulin levels, and skin tests were performed when possible. B-cell counts were low in all patients at the onset of DRESS, and natural killer (NK) cells were low in all cases except one. During recovery, B-cell numbers were within a normal range in five patients. In one, there was even a 10-fold increase in B-cell counts, although the level was mildly low after 3 months. NK-cell numbers were within a normal range in three patients. The mean numbers of B cells and NK cells were significantly higher in the second samples compared to the values on admission. Serum IgA and IgM levels were low in one patient. The drug provocation test was positive with cefotaxime in one patient. Viral serology, performed on five patients, was negative. A decrease in B-cell and NK-cell counts was the most consistent finding associated with the onset of antibiotic-induced DRESS in our patients. This immunologic alteration might be a useful predictor of DRESS development.

Drug reaction with eosinophilia and systemic symptoms (DRESS) or drug-induced hypersensitivity syndrome (DIHS) is a life-threatening drug reaction, characterized by rash, fever, lymphadenopathy, eosinophilia, and visceral involvement. Sulphonamides, aromatic anticonvulsants, allopurinol, and nevirapine are most often implicated as causal agents (1–2). The exact pathogenesis of this syndrome remains unknown. Recently, the role of human herpesvirus 6 (HHV-6) reactivation as a possible etiologic factor in DRESS has been suggested (3–6). It has been proposed that drug (metabolites)-induced hypogammaglobulinemia and B-cell depletion lead to an environment in which HHV-6 reactivates (8). Recently, a decrease in natural killer (NK)-cell numbers (9) or function (9) have been suggested as being the basis for the subsequent reactivation of HHV-6 and the development of DRESS. Additionally, Blanca et al. (10) demonstrated that NK cells could induce the differentiation of B cells into Ig-producing cells.

In our previous case report, a low number of NK cells and B cells were found to be an important contributor to the

development of DRESS, associated with the reactivation of HHV-6 (11). In this study, we report six cases who developed DRESS during antibiotic treatment, with the subsequent aim of investigating immunologic changes in antibiotic-induced DRESS.

Patients and methods

Patients

Six children – four males and two females – aged 15 months to 10 yr, who had developed DRESS during antibiotic treatment and were monitored for at least 3 months, were included in the study. The patients were admitted to our Pediatric Allergy and Pulmonology department between March 2006 and June 2009. The clinical diagnoses found different types of pulmonary infections these cases: One patient with Down syndrome additionally had severe sepsis and probable immunodeficiency. Antibiotics administered to these

children were cefotaxime, with or without vancomycin, and amikacin. DRESS diagnosis was based on criteria from Kardaun et al. (12) in the RegiSCAR study group and includes at least three of the following five criteria: Acute skin rash, fever ($>38^{\circ}\text{C}$), enlarged lymph nodes in at least two sites, involvement of at least one internal organ, and blood count abnormalities [lymphocyte level above or below the laboratory limits, eosinophil level above laboratory limits (in percentage or absolute count), and platelet level below laboratory limits]. In our patients, eosinophilia was defined as more than 10% of total white blood cells (if leukocytes $<4000/\text{mm}^3$) or $>700/\text{mm}^3$ (12).

Shiora et al. (2) have recently established a set of criteria for the diagnosis of DRESS and suggested the determination of HHV-6 reactivation as an important criteria for the diagnosis of DRESS/DIHS. In our study, we were unable to determine HHV-6 reactivation because of cost and technical issues. Some of the criteria suggested by Shiora et al. for this syndrome were not present at admission. In their series of patients from Japan, the drugs responsible for the development of DIHS were limited to eight drugs in the vast majority of patients. They included carbamazepine, phenytoin, phenobarbital, zonisamide, mexiletine, dapsone, sulfasalazine, and allopurinol. However, in our cases, the syndrome was induced by antibiotics. Therefore, all the criteria proposed by Shiora et al. does not apply to our cases.

Because this is a descriptive, non-interventional study, the project did not require approval by an institutional review board. The characteristics of the cases are summarized in Table 1.

Administration of vancomycin was the first drug to be stopped in three cases (cases 1, 2, and 4) in the first 1 or 2 days of skin eruptions; as no improvement was noted, cefotaxime was stopped in days 2–4. In case 3, all medications were immediately stopped on the day of the skin eruptions. In cases 5 and 6, cefotaxime was stopped on the second day of the onset of the symptoms. Hydroxyzine was administered for extreme pruritus. For high fever, acetaminophen was administered.

Discontinuation of cefotaxime led to a gradual reduction of body temperature and the severity of the rash in all of the cases. Fever subsided 1–7 days after the discontinuation of the medications. The eruptions gradually resolved in 6–10 days, and mild desquamation was observed in four cases (cases 1, 2, 4, and 5). In case 4, the rash was not accompanied by high fever. In case 6, a mild maculopapular eruption was observed on the hands and feet, 1 day after the axillary temperature returned to normal level.

Standard laboratory tests

A complete blood count with white blood cell differential was performed on all of the patients using automated hematology analyzers (Beckman Coulter LH 780 Analyzer; Beckman Coulter, Galway, Ireland). Other laboratory tests, such as erythrocyte sedimentation rate (ESR), C-reactive protein, aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase, γ -glutamyl transpeptidase,

blood urea nitrogen, and creatinine and urine analyses were also performed.

Flow cytometry

Lymphocyte subsets were identified by fluorescence-assisted cell sorting (FACS) by using a three-color Epics XL Coulter Flow Cytometer with System II software. Three milliliter of blood was inserted into EDTA tubes for FACS analysis. The first blood samples were obtained within 4 days from the initiation of the skin manifestations, and the tests were repeated 3–4 months later and 8–19 months later. Results were compared to reference limits (13). Verbal informed consent was obtained from the parents before the procedure.

Viral serology

Virological investigations were performed to examine the possibility of viral infections in five cases. Samples of the patients' sera were tested for viral hepatitis (types A, B, and C), the Epstein–Barr virus (EBV), rubella, parvovirus B19, cytomegalovirus, and human immunodeficiency virus (HIV) infection when possible. We could not investigate human herpesvirus 6 (HHV-6) DNA in these cases.

Serum immunoglobulin levels

Total serum IgM, IgG, and IgA levels were measured by a nephelometric assay (Beckman Coulter). Total serum IgE was measured by a solid-phase, two-site chemiluminescent enzyme immunometric assay (Diagnostic Products Corporation, Los Angeles, CA, USA), according to the package insert. Results were compared with reference limits (14).

Skin tests

Skin tests were performed in case 1 and case 2, using intravenous (parenteral) preparations of vancomycin HCl and cefotaxime. Reconstitution and further dilutions were carried out under sterile conditions no more than 2 h before administration. A solution of vancomycin (50 mg/ml, full-strength concentration) (16) and cefotaxime (10 mg/ml) (15–16) were used as stock solutions. Then sequential dilutions for vancomycin (0.1–1–10 $\mu\text{g}/\text{ml}$) and cefotaxime in a 0.9% saline (0.1–1–10 mg/ml) were prepared.

In case 1, skin tests with vancomycin and cefotaxime were performed on the 19th day after the onset of the eruptions. In case 2, skin tests with vancomycin and cefotaxime were performed on the 23rd and 24th day after the onset of the eruptions. None of the patients were under any other treatment that might have modified the skin tests. Epinephrine, antihistamines, and potential transfer to the Emergency Department were immediately available on site in case there was any suspicion of a systemic reaction. Parents received information about the possible risks of skin tests, and written informed consent was obtained from the parents for each patient before the procedure.

Table 1 Clinical data for the patients

Case no.	1	2	3	4	5	6
Age/sex	10 yrs/M	7 yrs/M	15 months/M	3 yrs/M	4 yrs/F	4 yrs/F
Treatment	23rd day	17th day	16th day	23rd day	15th day	15th day
Antibiotic	Cefotaxime and vancomycin	Cefotaxime and vancomycin	Cefotaxime, vancomycin, and amikacin	Cefotaxime and vancomycin	Cefotaxime	Cefotaxime
Fever	37.9°C	38.7°C	38.2°C	36.9°C	39°C	39°C
Facial edema	+	+	–	+	+	+
Lymphadenopathy	Right cervical lymphadenopathy	Bilateral occipital and submandibular lymphadenopathy	Right pre-auricular, post-auricular, and cervical conglomerate lymph node enlargement, and left cervical lymphadenopathy	–	–	Right submandibular and axillary lymphadenopathy
Rash	Maculopapular eruption	Maculopapular eruption	Maculopapular eruption	Maculopapular eruption	Maculopapular eruption	Maculopapular eruption
Treatment	Vancomycin stopped on day 1 Cefotaxime stopped on day 2 Hydroxyzine, acetaminophen Fever: 4 days Eruption: 6 days	Vancomycin stopped on day 1 Cefotaxime stopped on day 2 Hydroxyzine, acetaminophen Fever: 3 days Eruption: 10 days	Vancomycin and cefotaxime stopped on the day of eruption Acetaminophen Fever: 1 day Eruption 6: days	Vancomycin stopped on day 2 Cefotaxime stopped on day 4 of the eruption Hydroxyzine Eruption: 6 days	Cefotaxime stopped on day 2 of the onset of the symptoms Hydroxyzine, acetaminophen Fever: 7 days Eruption: 6 days	Cefotaxime stopped on day 2 of the onset of the symptoms Hydroxyzine, acetaminophen Fever 2: days A mild eruption appeared 1 day after the axillary temperature returned to normal
Duration of symptoms after discontinuation of cefotaxime or cefotaxime and vancomycin together						

Intradermal tests (IDT) was performed as described (17), starting with the highest dilution. European Network for Drug Allergy (ENDA) guidelines suggest that the highest concentrations recommended for both prick and intradermal testing with cephalosporins is 2 mg/ml (18). On the other hand, Empedrad et al. (15) reported a non-irritating skin test concentration for cefotaxime as 10 mg/ml, and Demoly et al. (16) suggested the highest concentration for both prick and intradermal testing with cephalosporins should be 10 mg/ml. In our two cases, we used a higher concentration for cefotaxime skin testing.

For patch testing, Finn Chambers on adhesive tape with a diameter of 12 mm were used. A filter paper disk was placed in each chamber. A drop of vancomycin (50 mg/ml), cefotaxime (10 mg/ml), and a normal saline solution were put on the filter paper sufficient to just soak the disk. Each Finn Chamber on an adhesive tape was then applied to normal skin on the child's upper back. The occlusion time was 48 h, and results were read 15 min after removing the cups, and then again at 48 and 72 h (17, 19).

There is a consensus of opinion that skin tests should be performed after a time interval which allows for resolution of clinical symptoms, and clearance from the circulation of the incriminated drugs and anti-allergic medications. However, it is not known whether the reactivity might be higher (e.g., cellular hyper-reactivity) or lower (e.g., initial histamine depletion of mast cells or tolerance) if skin tests are performed directly after the reaction (within the next few days). Thus, many groups carry out tests after a minimal time interval of, for example, 3 wk, but not after more than 3 months, if possible (17). In our case 1, skin tests were performed on the 19th day after the onset of the eruptions, as we needed to ascertain the responsible drug.

Drug provocation test

In case 1, a drug provocation test with cefotaxime was made 14 days after the resolution of the symptoms. Before the test, parents received information about the possible risks of drug provocation test (DPT), and written informed consent was obtained from them. The drug was administered in doses of 10, 25, 50, 100, 500 mg, and 1 g every 30 min, under close observation (20). All challenge phases were performed in a specialized hospital unit, where appropriate medication and resuscitation equipment was directly available.

Re-administration of drugs that caused severe non-IgE-mediated reactions is generally contraindicated if it caused a severe non-IgE-mediated reaction such as SJS, TEN, or exfoliative dermatitis. Rare exceptions to this may exist, such as treatment of a life-threatening illness, in which case the benefit of treatment outweighs the risk of a potentially life-threatening reaction (21). In our case, a cautious DPT was performed to find which drug induced the reaction.

Results

The results of the white blood count (WBC) and differential and other standard laboratory tests are shown in Table 2.

The mean ($\pm$ s.d.) of the WBC count was $4415 \pm 2243.87/\text{mm}^3$ (2700–8800) (N: 4600–10,200); the eosinophil count was $357.83 \pm 223.37/\text{mm}^3$ (range 147–704) [N: $<700/\text{mm}^3$, or $>10\%$ of total WBCs (if leukocytes $<4000/\text{mm}^3$)]; ESR was 34 ± 9.27 mm/h (range 18–42) (N: 8–12); AST was 76.33 ± 49.91 U/l (range 43–176) (N: 15–41); ALT was 57.17 ± 45.19 U/l (range 25–141) (N: 14–54).

The absolute number of $\text{CD3}^+/\text{CD4}^+$, $\text{CD3}^+/\text{CD8}^+$, CD19^+ , and $\text{CD3}^+/\text{CD16}^-/\text{CD56}^-$ (NK cells), and the $\text{CD4}^+/\text{CD8}^+$ ratio in initial and subsequent samples are shown in Table 3. Descriptive data are given in Table 4. The mean ($\pm$ s.d.) of CD19^+ cells, NK cells, and the $\text{CD4}^+/\text{CD8}^+$ ratio was 67.17 ± 58.90 , 139.33 ± 88.06 , and 1.17 ± 0.44 cells/ml, respectively. In the initial blood samples, all of our patients exhibited a low number of CD19^+ cells. The $\text{CD4}^+/\text{CD8}^+$ cell ratio was low in two of the cases. The absolute number of NK cells was low in all of the cases, except in case 3 (Table 3).

The mean ($\pm$ s.d.) of CD19^+ cells, NK cells, and the $\text{CD4}^+/\text{CD8}^+$ ratio in the second samples (3–5 months later) was 602.33 ± 440.80 , 340 ± 166.39 , and 1.41 ± 0.40 cells/ml, respectively. In the second samples, all of the patients had higher numbers of CD19^+ cells, reaching a normal range. However, in case 3, even though there was a 10-fold increase in B-cell counts, the level was still mildly low after 3 months. The $\text{CD4}^+/\text{CD8}^+$ cell ratio was within a normal range in all of the cases. NK-cell numbers reached a normal range in three patients (case 1, 2, and 4) in 3–4 months. However, in cases 3, 5, and 6, the absolute number of NK cells was low in the second blood samples. The mean numbers of CD19^+ cells, CD4^+ cells, and NK cells were significantly higher in the second samples compared to the values on admission ($p = 0.04$, $p = 0.02$, and $p = 0.03$, respectively) (Table 4).

Subsequent blood samples were obtained 7–19 months after the initiation of DRESS in all of the cases; except in case 3, we could not reach this patient because of the change of family address and phone numbers. The mean ($\pm$ s.d.) of CD19^+ cells, NK cells, and the $\text{CD4}^+/\text{CD8}^+$ ratio in the final samples was 582.20 ± 223.64 , 223.40 ± 99.94 , and 1.37 ± 0.26 cells/ml, respectively. In all of the cases, in the third blood samples (in case 6, additionally in the fourth), the absolute number of CD19^+ cells, and the $\text{CD4}^+/\text{CD8}^+$ cell ratio were within the normal range. NK-cell numbers were still in a low range in cases 5 and 6, 18 and 14–19 months after the onset of symptoms (Table 3).

Viral serology

Serologic test results for viral hepatitis (types A, B, and C), mumps, rubella, parvovirus B19, and HIV were negative in the cases of those tested. Anti-cytomegalovirus IgM, IgG antibodies, and the Epstein-Barr virus capsid antigen-specific IgM and IgG antibodies did not indicate primary infection or reactivation. Human herpesvirus 6 (HHV-6) DNA was not investigated in these cases because of cost and technical issues.

Table 2 Laboratory tests

Case no.	1	2	3	4	5	6
WBC (/mm ³)*	8800 (3700–11,100)	2700 (5000–15,500)	4600 (4400–12,900)	3090 (4000–10,400)	3500 (4000–10,400)	3800 (4000–10,400)
Eosinophils (/mm ³)	704	540	230	169	147 (1722 on day 9)	357
Lymphocytes (/mm ³)	3344	1890	1610	865	1200	2044
Platelets (/mm ³) (N:150,000–450,000/mm ³)	345,000	234,000	396,000	247,000	125,000	98,000
Erythrocyte sedimentation rate (N: 8–12 mm/hr)	41	18	29	34	40	42
C-reactive protein, (mg/dl) (N: 0–1 mg/dl)	0.1	2.1	0.4	1.73	6.79	0.73
Aspartate aminotransferase (U/l) (N:15–41 U/l)	46	176	68	43	58	67
Alanine aminotransferase (U/l) (N:14–54 U/l)	77	141	29	25	32	39
Lactate dehydrogenase (U/l) (N: 0–450 U/l)	ND	ND	ND	615	830	ND
γ -glutamyl transpeptidase (U/l) (N: 0–38 U/l)	ND	ND	ND	ND	22	9
Alkaline Phosphatase (U/l) (N: 80–644 U/l)	ND	ND	ND	116	239	ND
IgG (mg/dl)†	1390 (608–1572)	986 (633–1280)	1030 (424–1051)	ND	850 (463–1236)	ND
IgA (mg/dl)†	147 (52–236)	68 (33–202)	9.15 (14–123)	ND	73.1 (25–154)	ND
IgM (mg/dl)†	75.1 (52–242)	56.4 (48–207)	40 (48–168)	ND	192 (43–196)	ND
IgE (IU/ml)†	92.8 (0.98–570.6)	42 (1.03–161.3)	3.24 (0.31–29.5)	ND	235 (1.07–68.9)	ND

ND, not done. *Normal numbers of white blood cells in healthy Turkish children within the same age group (5th and 95th percentiles) in parenthesis. †Serum Ig levels in healthy children in parenthesis (Ref. no. 15).

Serum immunoglobulins

Serum immunoglobulins were within a normal range in all of the cases, except in case 3. In case 3, the level of serum IgA was 9.15 mg/dl (normal: 14–123), and the level of serum IgM was 40 mg/dl (normal: 48–168). In case 4 and case 6, serum immunoglobulin levels were not determined (Table 2).

Skin tests

Intradermal tests and skin patch tests were performed in cases 1 and 2. The results were all negative.

Drug provocation test

Maculopapular eruptions with a tendency to confluence were observed all over the body 2 h after administering the final drug dose in case 1. We did not observe other symptoms of DRESS, because we did not continue the treatment with the same drug. We could not perform an *in vitro* assay as a confirmatory test.

Discussion

Drug reaction with eosinophilia and systemic symptoms is a life-threatening, drug-induced hypersensitivity syndrome. Aromatic anticonvulsants, sulfonamides, allopurinol, and nevirapine are most often implicated as causal agents (1–2). Few studies have reported vancomycin-induced drug hypersensitivity reaction. However, DRESS is very rarely reported as a result of vancomycin (22–25) and cephalosporins such as cefuroxime and ceftriaxone (26–28). Only in one case report was DRESS secondary to cefotaxime presented (29).

Drug reaction with eosinophilia and systemic symptoms was diagnosed in our cases according to the presence of at least three of the following five criteria: Skin rash, fever (> 38°C), enlarged lymph nodes in at least two sites, involvement of at least one internal organ, and blood count abnormalities (12). In four cases, DRESS developed during treatment with vancomycin and cefotaxime. We stopped vancomycin first in three of the cases. As no resolution of the symptoms was observed, cefotaxime was then stopped. Discontinuation of cefotaxime led to a gradual reduction in body temperature and the severity of the rash.

Table 3 Absolute numbers of peripheral blood lymphocytes

Case no.	Counts, cells/ml																	
	Initial* Day 2	Month 4	Month 8	Initial Day 2	Month 3	Month 7	Initial Day 4	Month 3	Month 9	Initial Day 1	Month 4	Month 18	Initial Day 4	Month 5	Month 14	Month 19		
1				2	3		3	4	5	6								
CD3 ⁺ /CD4 ⁺ T helper T cells [†]	1057 [1000 (500–2700)]	1773	1104	782 [1000 (500–2700)]	1067	743	597 [2100 (700–4500)]	1223	314 [1500 (600–2000)]	2157	1601	469 [1500 (600–2000)]	1068	1476	886 [1500 (600–2000)]	1589	1672	1267
CD3 ⁺ /CD8 ⁺ T cytotoxic T cells [†]	1615 [800 (300–2100)]	1014	978	567 [800 (300–2100)]	863	513	573 [1000 (400–3200)]	1096	376 [700 (300–1300)]	2076	1386	401 [700 (300–1300)]	837	1111	464 [700 (300–1300)]	777	871	720
CD19 ⁺ (B cells) [†]	167 [500 (200–2200)]	347	361	46 [500 (200–2200)]	440	368	17 [1400 (500–3600)]	399	13 [700 (300–1200)]	1497	894	56 [700 (300–1200)]	485	630	104 [700 (300–1200)]	446	565	658
CD37/CD16 ⁺ /CD56 ⁺ (NK cells) [†]	125 [500 (200–900)]	440	310	156 [500 (200–900)]	443	223	293 [500 (200–1300)]	385	67 [300 (200–1200)]	505	332	43 [300 (200–1200)]	93	97	152 [300 (200–1200)]	174	138	155
	0.65	1.75	1.13	1.38	1.24	1.45	1.04	1.09	0.84	1.04	1.16	1.17	1.28	1.33	1.91	2.04	1.92	1.76

CD4/CD8 normal ratio: >1. *Test time after the onset of rash. †Absolute numbers of peripheral blood lymphocyte subsets in healthy Turkish children within the same age group [medians (5th and 95th percentiles)] in parenthesis.

The value of skin tests is not clear in DRESS. Performing IDT only when patch tests give negative results is generally advised. Even though the response to drug discontinuation suggests that cefotaxime was responsible for the development of DRESS, we performed skin tests in our first two cases (case 1 and case 2), using intravenous preparations of vancomycin HCl and cefotaxime. As the skin tests results were negative, a cautious drug provocation test with cefotaxime was carried out only in one case (case 1) 14 days after the resolution of the symptoms. The positive response to the DPT confirmed that cefotaxime was responsible for the development of DRESS.

Re-administration of drugs that caused severe non-IgE-mediated reactions is generally contraindicated if it caused a severe non-IgE-mediated reaction. However, treatment of a life-threatening illness, in which case the benefit of treatment outweighs the risk of a potentially life-threatening reaction, might be an exception (21). In our case, a cautious DPT was performed to ascertain which drug induced the reaction. After confirming that cefotaxime was the responsible drug causing DRESS, we decided to continue our treatment with another drug not cross-reacting with cefotaxime.

Another important finding in DRESS is the prolonged clinical symptoms after withdrawal of the causative drug (2). In one of our cases (case 5), eosinophilia was more prominent on day 9 after the onset of DRESS, and in one case (case 6), a mild eruption appeared 1 day after the axillary temperature returned to normal after the discontinuation of the drug.

The exact pathogenesis of DRESS remains unknown. The metabolism of the parent drug to chemically reactive metabolites is thought to be important in initiating the cascade of events leading to DRESS (1). Recently, the role of HHV-6 reactivation as a possible etiologic factor in DRESS has been suggested (3–6). Reactivation of HHV-6 from latently infected peripheral blood mononuclear cells (PBMCs) requires T-cell activation. Tohyama et al. (4) suggested T-cell activation caused by reactive metabolites as the primary step for the development of DRESS. However, many adverse drug reactions via T-cell activation do not always develop into DRESS. Alternatively, Kano et al. (7) stated that drug (metabolites)-induced hypogammaglobulinemia and B-cell depletion lead to an environment in which HHV-6 reactivates. Recently, Inaoka (9) proposed that the intrinsic dysfunction of NK cells to express CD122 is primarily responsible for the drug-induced decrease in B cells, and the subsequent reactivation of HHV-6, thereby causing excessive activation of antiviral CD8⁺ T-cell responses in compensation for dysfunctional NK cells. In our previous case report, the low numbers of NK cells and B cells were found to be an important contributor to the development of phenytoin-induced DRESS associated with reactivation of HHV-6 (11). Additionally, Blanca et al. (10) demonstrated that NK cells could induce the differentiation of B cells into Ig-producing cells.

In the present study, in which we report on our cases with antibiotic-induced DRESS, the most consistent finding was a low number of B cells at the onset of symptoms. In one case

Table 4 Descriptive data on initial number of peripheral blood lymphocytes and changes during the follow-up period

	Initial (days 1–4)		Months 3–5		Months 7–19	
	Mean $\pm$ SD Median	Range	Mean $\pm$ SD Median	Range	Mean $\pm$ SD Median	Range
CD3 ⁺ /CD4 ⁺ cells	684.17 $\pm$ 275.76* 689.5	314–1057	1479.5 $\pm$ 438.73* 1406	1067–2157	1238.2 $\pm$ 336.24 1267	743–1267
CD3 ⁺ /CD8 ⁺ cells	666 $\pm$ 472.06 515.5	376–1615	1110.5 $\pm$ 487.65 938.5	777–2076	941.6 $\pm$ 339.23 978	513–1386
CD19 ⁺ cells	67.17 $\pm$ 58.90** 51	13–167	602.33 $\pm$ 440.80** 443	347–1497	582.2 $\pm$ 223.64 630	361–894
CD3 ⁺ /CD16 ⁺ /CD56 ⁺ (NK cells)	139.33 $\pm$ 88.06*** 138.5	43–293	340 $\pm$ 166.39*** 412.5	93–505	223.4 $\pm$ 99.94 223	97–310
CD4 ⁺ /CD8 ⁺	1.17 $\pm$ 0.44 1.105	0.65–1.91	1.41 $\pm$ 0.40 1.26	1.04–2.04	1.37 $\pm$ 0.26 1.33	1.13–1.76

*p = 0.02; **p = 0.04; ***p = 0.03.

(case 3), serum IgA and IgM levels were also low. In this case, low IgA and IgM concentrations together with the low CD4 T-cell counts (at all times) and low B-cell counts (at all times) suggested immunodeficiency associated with Down syndrome. In five cases, the absolute number of NK cells was also low at the onset of symptoms. Additionally, NK-cell numbers were still low, even 18 and 14–19 months after the acute onset of DRESS in cases 5 and 6. These results support the importance of NK cells in the pathogenesis of antibiotic-induced DRESS. Although this study did not evaluate the function of NK cells, we believe that investigating their function might give us a better understanding of the immune mechanisms in DRESS.

A striking feature we observed was the normal white cell counts in our patients, and there was no neutrophilia during the onset of DRESS, even though they were under treatment because of life-threatening infections, such as necrotizing pneumonia, pneumonia with pleural effusion, severe sepsis, etc. Peyriere et al. (30), though, did report neutropenia among observed hematologic abnormalities in patients diagnosed with DRESS.

In this study, we could not investigate HHV-6 DNA by PCR in PBMCs. Recently, it was reported that reactivation of other HHVs besides HHV-6 might also be involved in the development of DRESS (2–6). However, other available virological tests also gave negative results in our patients. We

considered that B-cell dyscrasia might have produced a false negative serology result.

Recent reports suggest that a decrease in B- and NK-cell counts can be associated with HHV-6 reactivation and the subsequent onset of DRESS. As compensation for low NK-cell numbers, there is excessive activation of antiviral CD8⁺ T-cell responses. CD8⁺ T cells could be cross-reactive with multiple antigens, causing severe clinical illness involving multiple organs in DRESS. In our previous report, we observed a high number of CD8⁺ T cells at the onset of symptoms with a low CD4⁺/CD8⁺ ratio (11). In the present study, we observed a low CD4⁺/CD8⁺ ratio in two of our cases. We believe the elevation of CD8⁺ T-cell numbers would have been more pronounced if we had continued longer with the antibiotic.

We conclude first that a decrease in B-cell and NK-cell counts was the most consistent finding associated with the onset of antibiotic-induced DRESS in our patients. This immunologic alteration might be a useful predictor of the development of DRESS. We also conclude that immunophenotyping of peripheral blood lymphocytes would be useful for the early detection of this syndrome. We would recommend that this immunologic alteration be included in the diagnostic criteria for this condition. We believe that our observation will open up a new area in investigating the pathogenic role of B- and NK-cell depletion in DRESS.

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