

# Specific IgE toward Allergenic Molecules Is a New Prognostic Marker in Patients with Sézary Syndrome

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## Key Words

Sézary syndrome • Cutaneous T cell lymphoma • Specific IgE • Allergen microarray • Survival rate • CD60+CD49–CD4+ T cell phenotype

## Abstract

**Background:** Sézary syndrome (SS) is the aggressive leukemic form of cutaneous T cell lymphoma characterized by erythroderma, the presence of a malignant circulating T memory cells with skin homing potential, and the ability to produce a variety of Th2 soluble factors, such as IL-4 and IL-5. We measured total and specific IgE in SS patients as a further parameter of a Th2-skewed immune system, and studied their clinical impact. **Methods:** Specific IgE production in a cohort of 55 SS patients was evaluated by the molecule-based ISAC microarray system. We then evaluated survival times and the hazard ratios in this cohort by Kaplan-Meier and Cox methods. **Results:** Twenty-four (43.6%) SS patients had specific IgE to both environmental and food allergens. For survival analysis, patients found positive to at least one allergen were defined as IgE+. By comparing IgE+ versus IgE– we found a significant difference in the median survival times, 2.9 versus 8.9 years ( $p < 0.001$ ). Conversely, no survival

difference could be observed when total IgE levels were considered. IgE+ patients had higher levels of CD60+CD49–CD4+ T cells, which also represent another worse prognostic index recently identified. **Conclusion:** SS patients had specific IgE to both environmental and food allergens. IgE+ SS patients had a significant lower survival rate. High levels of CD60–CD49+CD4+ T cells associated with an IgE– phenotype allow the identification of a restricted group of long survivor SS patients. Therefore, specific measurement of IgE seems to be useful in discriminating survival.

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## Introduction

The presence of a measurable circulating clonal T cell population is one of the hallmarks of Sézary syndrome (SS), an extranodal uncommon variant of non-Hodgkin lymphoma of T cell origin with primary cutaneous involvement [1–5]. It has been suggested that the malignant CD4+ T cell in SS belongs to the Th2 type as shown by the IL-4 and IL-5 overproduction [6–10], although Th1-skewed cases have been described [11]. The Th2-skewed profile of the circulating clonal T cell population could

explain the immunodeficiency [12], the presence of eosinophilia [13] and the elevated IgE levels [14, 15], often observed in SS [16]. Recently, significantly higher levels of serum IgE and specific IgE positivity were described in a cohort of Turkish cutaneous T cell lymphoma (CTCL) patients [17].

There is an ongoing debate about the presence of a direct relationship between chronic dermatoses, atopy and subsequent CTCL development. Several case reports of the onset of CTCL in patients affected by atopic dermatitis (AD) seem to support the possibility of such an association [18–22], but other epidemiological studies have shown negligible evidence of a causal relationship or even a decreased risk [23, 24]. Surprisingly, none of the above mentioned studies evaluated the presence and relevance of a specific IgE response in CTCL cases. As a consequence, the conflicting results about the possible link between AD and CTCL found in the literature could be merely due to a biased observation following the use of improper markers, being either the clinical phenotype or the generic total IgE overproduction.

Specific IgE response in AD could be directed to environmental or food allergens. In addition, the presence of specific IgE to self-antigens has been clearly shown in vitro and in vivo, but the clinical relevance of this immune recognition still represents another controversial issue [25]. Furthermore, the presence of a specific IgE response to staphylococcal enterotoxins on the skin of patients affected by AD, with disease-promoting effect and increased severity of the AD, has been widely reported [26–32]. Similarly, in SS a high rate of *Staphylococcus aureus* colonization, analogous to what is observed in AD, has been shown [33]. Cutaneous colonization by *S. aureus* could influence the disease activity and exacerbate chronic T cell expansion and cutaneous inflammation in CTCL, as well [34, 35].

The aims of this study were to evaluate the specific IgE reactivity to a large panel of native and recombinant allergenic molecules in SS patients and compare it with other non-neoplastic patients, and to address whether IgE reactivity is associated with a different prognosis in this cohort of Italian patients affected by SS.

## Patients and Methods

### Patients

Fifty-five patients affected by SS (67.3% male, mean age  $65.9 \pm 12.5$  years), recruited at the Istituto Dermopatico dell'Immacolata (IDI-IRCCS), Rome, Italy from April 2000 to May 2009, were followed up to the end of March 2010. The diag-

nosis of SS was established on the basis of characteristic erythroderma, diagnostic skin biopsy specimen and blood Sézary cell count according to the WHO classification [36]. Demographical (age and gender) and clinical data were recorded for all patients, as well as the date of the first SS diagnosis. Informed consent for blood sampling was obtained from patients at the first clinical examination. Thirty patients (54.6% male) died during the follow-up period. All patients were clinically evaluated every 6 months to set or modify therapeutic strategies. Patients were regularly studied in details for T cell receptor (TCR)-V $\beta$  domain expression using a comprehensive panel of 24 monoclonal antibodies (IO Test Beta Mark TCR-V $\beta$  Repertoire Kit) obtained from Beckman Coulter (Fullerton, Calif., USA) and for the expression of activation/adhesion molecules on the peripheral T cell clones such as CD7, CD26, CD49d and CD60 [37].

This study has been approved by the IDI-IRCCS Ethical Committee and informed consent was obtained in accordance with the Declaration of Helsinki.

We compared the frequency of IgE reactivity in SS patients to that observed among 82 age-matched subjects (27.7% male, mean age  $63.6 \pm 11.2$  years) affected by contact dermatitis, that is, a nonneoplastic dermatological disease. Thirty-seven (44.5%) patients affected by contact dermatitis were IgE reactive to at least one allergenic molecule, and thus acted as our dermatological control group. Four hundred forty-seven patients (68% male, mean age  $67.3 \pm 10.1$  years) affected by allergic respiratory disease such as rhinitis or asthma represented a further age- and gender-matched control group.

### Determination of Total IgE

The concentrations of total IgE were determined by using the CAP System according to the manufacture's instruction (Phadia, Uppsala, Sweden).

### IgE Microarray Assay

The comprehensive IgE sensitization profile of SS patients was investigated using the ImmunoCAP ISAC CRD103® allergen chip (Phadia Multiplexing Diagnostics, Vienna, Austria) providing a panel of 103 common allergens immobilized onto solid phase on a chemically modified microscopy slide [38]. A customized microarray was also set, containing further allergens, namely Der s 1, a mite allergen homologous of Der p 1 and Der f 1, staphylococcus enterotoxin A and B (Sta a SEA and Sta a SEB, respectively). The testing was performed following the manufacturer's assay protocol [39]. Briefly, glass microscopy slides, each bearing 4 allergen reaction sites, were placed into a humid chamber and 20  $\mu$ l of undiluted serum from each patient was applied to the reaction site for 2 h at RT. After incubation with patient's sera, slides were rinsed and washed with buffer solution in order to remove unbound serum IgE. After drying, allergen-specific IgE bound to the single allergens was detected with a secondary fluorescence-labeled anti-human IgE antibody after 1 h of incubation at room temperature, protected from light. After a second rinsing and washing procedure, fluorescence was measured using a commercial biochip reader (ScanArray Gx Microarray Analysis System; PerkinElmer Life and Analytical Sciences, Shelton, Conn., USA). Captured images were acquired as tagged image file format and imported into the ISAC software (Phadia Multiplexing Diagnostics). IgE values were expressed as ISAC

**Table 1.** Clinical and laboratory features of patients affected by SS, compared to patients affected by chronic eczema or allergic respiratory disease

|                                | SS                                   | Contact dermatitis               | Respiratory disease              |
|--------------------------------|--------------------------------------|----------------------------------|----------------------------------|
| SS patients studied            | 55                                   | 19                               | 20                               |
| Sex, male/female               | 37/18                                | 23/59                            | 304/143                          |
| Age, years                     | 65.93 ± 12.54                        | 63.6 ± 11.2                      | 67.3 ± 10.1                      |
| Overall median surviva, months | 45 ± 36                              | –                                | –                                |
| Median total IgE, kU/l         | 13                                   | 98                               | 175                              |
| 25th–75th percentile           | 5–61                                 | 65–201                           | 68–435                           |
| White blood cells, cells/μl    | 10,369 ± 6,758                       | 4,590 ± 1,034                    | 4,187 ± 951                      |
| Eosinophils, cells/μl          | 340 ± 434                            | 115 ± 116                        | 203 ± 279                        |
| Total lymphocytes, cells/μl    | 5,559 ± 5,445                        | 1,791 ± 709                      | 2,406 ± 582                      |
| Clonal T cell population, %    | 78.6 ± 16 (3,970 ± 4,840 cells/μl)   | –                                | –                                |
| CD3+CD4+, %                    | 76.5 ± 18.5 (4,499 ± 5,035 cells/μl) | 50.1 ± 13.1 (910 ± 403 cells/μl) | 44.6 ± 12.3 (964 ± 294 cells/μl) |
| CD3+CD8+, %                    | 7.6 ± 8.9 (231 ± 200 cells/μl)       | 26.1 ± 12.8 (464 ± 233 cells/μl) | 27.9 ± 16.1 (688 ± 608 cells/μl) |
| CD4/CD8 ratio                  | 37.14 ± 53.8                         | 2.5 ± 1.6                        | 2.3 ± 1.8                        |
| CD3+CD4+CD60+, %               | 40.5 ± 30.6 (1,814 ± 3,119 cells/μl) | 26.9 ± 29.7 (215 ± 279 cells/μl) | 15.1 ± 10.6 (152 ± 129 cells/μl) |
| CD3+CD4+CD26+, %               | 13.7 ± 20.1 (264 ± 383 cells/μl)     | 64.9 ± 25.9 (572 ± 334 cells/μl) | 72.8 ± 20.1 (734 ± 259 cells/μl) |
| CD3+CD4+CD7+, %                | 31.3 ± 31.8 (1,092 ± 1,899 cells/μl) | 73.8 ± 16.4 (654 ± 293 cells/μl) | 72.8 ± 20.1 (601 ± 245 cells/μl) |
| CD3+CD4+CD49d+, %              | 23.6 ± 25.6 (804 ± 1,420 cells/μl)   | 63.5 ± 27.7 (533 ± 371 cells/μl) | 65.5 ± 11.8 (640 ± 247 cells/μl) |

A significant difference ( $p < 0.0001$ ) was recorded among all the CD4+ T cell subset studied when compared to SS patients. Results are means ± STD unless otherwise specified.

standardized units (kU<sub>a</sub>/l) by interpolating the mean fluorescence value with a previously established reference curve [40, 41]. At the end of each run, data were exported to the InterAll software version 3.0 (Allergy Data Laboratories s.c., Latina, Italy) by a real-time connectivity, and stored in the patient's record together with demographical and clinical data from SS and all enrolled control patients.

#### Survival and Statistical Analyses

A time-to-event analysis was performed using the nonparametric Kaplan-Meier product-limit survival estimates, and differences between Kaplan-Meier survival curves were analyzed using the Mantel-Haenszel log-rank test. Statistical analyses were performed using SPSS version 13.0 (SPSS Inc., Chicago, Ill., USA). Median survival times, and the probabilities of surviving at 5 and 10 years after first observation were derived from the same curves [42].

The receiver operating characteristic curve analysis was used to identify the optimal cutoff values with the higher sensitivity and specificity for each variable of interest (total IgE, total lymphocytes, CD4+ T cells, TCR-Vβ clone, CD8+ T cells, CD4+ CD60+ T cells, CD4+CD26+ T cells, CD4+CD49d+ T cells and eosinophils) [43]. To estimate the possible independent role of the different factors of interest, we also applied the Cox proportional hazard method [44]. We, therefore, generated graphs of the log[–logS(t)] versus time, to check the necessary assumption of proportionality of risks [45].

## Results

We started to observe patients at the time they were referred to our center; therefore, all our testing has been performed in a cross-sectional way at the time of the SS diagnosis. The main clinical and laboratory features of the cohort of SS patients analyzed are shown in table 1 and are expressed as average (median values). SS patients were prevalently male (67.3%) and the mean age was 66 years. As expected, high levels of circulating clonal T cell were observed by mean of serial evaluation of TCR-Vβ repertoire in all patients (data not shown), associated with an aberrant expression of homing/activation molecules conferring to those cells a characteristic and unequivocal signature (CD4+CD60+CD7–CD26–CD49d–) (table 1) [46, 47].

#### Allergenic Molecule IgE Recognition in SS Patients

Twenty-four SS patients (43.6%) recognized at least 1 molecule among the 103 tested by means of the ISAC system, thus they were considered as IgE+. Similarly, 45.1% of the individuals affected by contact dermatitis were IgE+ as well. The IgE reactivity to the purified natural and recombinant components detected for each SS patient is shown in table 2. The reactivities most frequently

**Table 2.** IgE reactivity to purified and recombinant component expressed as kU<sub>a</sub>/l in 24 individuals affected by SS

|              | Patients |      |      |     |     |     |     |     |     |     |     |     |     |      |     |     |     |     |     |     |     |     |     |     |
|--------------|----------|------|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|              | 1        | 2    | 3    | 4   | 5   | 6   | 7   | 8   | 9   | 10  | 11  | 12  | 13  | 14   | 15  | 16  | 17  | 18  | 19  | 20  | 21  | 22  | 23  | 24  |
| Aln g 1.0101 |          |      |      |     |     |     |     |     |     | 0.4 |     |     |     |      |     |     |     |     |     |     |     |     |     |     |
| Ana c 2      |          |      |      |     |     |     |     |     |     |     |     |     |     |      |     |     | 0.8 |     |     | 0.4 |     |     | 0.1 |     |
| Ani s 1      |          |      |      |     |     |     |     |     |     |     |     |     |     | 1.8  | 0.5 |     |     |     |     |     |     |     |     |     |
| Api m 1      |          |      |      |     |     |     |     |     |     |     |     |     |     |      |     |     | 1.5 |     |     |     |     |     |     |     |
| Bet v 1.0101 |          |      |      |     |     |     |     |     |     | 4.0 |     |     |     |      |     |     |     |     |     |     |     |     |     |     |
| Bos d 6      |          |      |      |     |     |     |     |     |     |     | 0.5 |     | 0.6 |      |     |     | 0.5 |     |     | 0.5 |     |     |     | 0.5 |
| Cor a 1.0101 |          |      |      |     |     |     |     |     |     | 1.0 |     |     |     |      |     |     |     |     |     |     |     |     |     |     |
| Cor a 1.0401 |          |      |      |     |     |     |     |     |     | 0.0 |     |     |     |      |     |     |     |     |     |     |     |     |     |     |
| Cor a 8      |          |      |      |     |     |     |     |     |     |     |     |     |     |      |     |     |     | 0.5 |     |     |     |     |     |     |
| Cup a 1      |          |      |      |     |     |     |     |     |     | 8.6 |     | 1.6 |     | 20.1 |     |     |     |     | 1.8 |     |     |     |     |     |
| Cyn d 1      |          | 1.4  |      |     |     |     | 0.7 |     |     |     |     | 0.5 |     |      |     |     | 1.1 |     |     |     |     | 2.0 |     |     |
| Der f 1      | 11.7     | 2.4  | 1.7  | 0.2 | 0.0 |     |     |     |     |     |     |     |     |      |     |     |     |     |     |     |     |     |     |     |
| Der f 2      | 57.8     | 86.9 | 23.8 |     |     |     |     |     |     | 1.7 |     |     |     |      |     | 1.9 |     |     |     |     | 2.5 |     |     |     |
| Der p 1      | 15.5     | 6.6  | 2.5  | 0.1 | 0.0 |     |     |     |     |     |     |     |     |      |     |     |     |     |     |     |     |     |     |     |
| Der p 2      | 53.8     | 66.8 | 18.8 |     |     |     |     |     |     | 2.3 |     |     |     |      |     |     |     |     |     |     | 3.7 |     |     |     |
| Der s 1      | 8.4      | 1.1  | 0.5  |     |     |     |     |     |     |     |     |     |     |      |     |     |     |     |     |     |     |     |     |     |
| Mal d 1.0108 |          |      |      |     |     |     |     |     |     | 1.6 |     |     |     |      |     |     |     |     |     |     |     |     |     |     |
| Ole e 1      |          |      |      |     |     |     |     |     |     | 0.5 |     |     |     |      |     |     |     |     |     |     |     |     |     |     |
| Par j 2      |          |      |      |     |     |     | 1.1 | 2.3 | 0.4 | 1.5 | 2.4 |     |     | 48.0 |     |     | 9.4 |     |     |     |     |     |     | 2.4 |
| Phl p 1      |          | 3.4  |      |     |     |     | 3.0 |     |     |     |     | 0.3 |     |      |     |     |     |     |     |     | 1.2 |     |     |     |
| Phl p 2      |          | 1.4  |      |     |     |     | 3.6 |     |     |     |     | 0.3 |     |      |     |     |     |     |     |     | 5.3 |     |     |     |
| Phl p 4      |          |      |      |     |     |     |     |     |     |     |     | 0.2 |     |      |     |     |     |     |     |     |     |     |     |     |
| Phl p 5      |          | 2.6  |      |     |     |     |     |     |     |     |     |     |     |      |     |     |     |     |     |     |     |     |     |     |
| Phl p 7      |          | 2.6  |      |     |     |     |     |     |     |     |     |     |     |      |     |     |     |     |     |     |     |     |     |     |
| Phlp 11      |          |      |      |     |     |     |     |     |     |     |     | 0.2 |     |      |     |     |     |     |     |     |     |     |     |     |
| Pla a 2      |          |      |      |     |     |     |     |     |     |     |     |     |     |      |     |     | 1.1 |     |     |     |     |     |     |     |
| Pru p 1.0101 |          |      |      |     |     |     |     |     |     | 1.1 |     |     |     |      |     |     |     |     |     |     |     |     |     |     |
| Pru p 3      |          |      |      |     |     |     |     |     |     |     |     |     |     |      |     |     | 0.5 |     |     |     |     |     |     |     |
| Ses i 1      |          |      |      |     |     |     |     |     |     |     |     |     |     |      |     |     |     |     |     |     |     |     | 0.3 |     |
| Sta a SEA    |          |      |      |     |     | 0.1 |     |     |     |     |     |     |     |      |     |     |     |     |     |     | 0.1 |     |     |     |
| Sta a SEB    | 0.1      |      | 2.5  |     |     | 1.3 |     |     |     |     |     |     |     |      |     |     |     |     | 2.5 |     | 0.3 | 0.5 |     |     |

found were those for the house dust mite allergen Der f 2 and the Parietaria pollen allergen Par j 2, both recognized by 7 distinct patients (29.2% of the IgE+). Six patients (25%) showed IgE reactivity for the Sta a SEB, none to Sta a SEA.

A higher prevalence of Sta a SEB IgE recognition ( $p = 0.023$ ) and a lower prevalence of IgE to cypress pollen Cup a 1 ( $p = 0.018$ ) were recorded in SS patients compared to individuals with contact dermatitis or respiratory allergic diseases (table 3). All other molecules studied were similarly ranked for prevalence in the different groups of patients (table 3).

Significant differences in total IgE levels were recorded comparing IgE+ patients to the IgE negative subset by means of Mann-Whitney U test ( $p = 0.05$ ). The IgE median value, in fact, was higher in the reactive population (61.8 kU/l, range 13–87), when compared to the nonreactive subset (7 kU/l, range 4–17). We then sought to verify if other markers of the peripheral malignant T cell clone, listed in table 1, were differently distributed among IgE+ and IgE– patients. Among these were also Th2 markers such as CD60 that are known effectors of allergic response. Then we compared specific subsets of circulating CD4+ cells between IgE+ and IgE–: interestingly, we found a significantly higher number of CD4+CD60+ T

**Table 3.** Prevalence (in percent) of allergenic molecule-based specific IgE reactivity in patients affected by SS, compared to patients affected by contact dermatitis and the general age-matched allergic population affected by respiratory diseases

| Allergens | SS (n = 24) | Contact dermatitis (n = 37) | Respiratory diseases (n = 447) |
|-----------|-------------|-----------------------------|--------------------------------|
| Der f 2   | 29.2 (1)    | 18.9 (8)                    | 24.1 (5)                       |
| Par j 2   | 29.2 (1)    | 35.1 (3)                    | 25.3 (4)                       |
| Der p 2   | 25 (3)      | 18.9 (8)                    | 17.9 (7)                       |
| Sta a SEB | 25 (3)      | 2.4 (65)                    | 3.3 (33)                       |
| Cyn d 1   | 20.8 (5)    | 29.7* (5)                   | 26.4* (3)                      |
| Der f 1   | 20.8 (5)    | 13.5 (12)                   | 10.3 (12)                      |
| Der p 1   | 20.8 (5)    | 10.8 (15)                   | 9.4 (14)                       |
| Bos d 6   | 16.6 (8)    | 8.1 (17)                    | 2.5 (37)                       |
| Cup a 1   | 16.6 (8)    | 45.9** (1)                  | 37.3 (1)                       |
| Phl p 1   | 16.6 (8)    | 35.1 (4)                    | 28.2 (2)                       |
| Phl p 2   | 16.6 (8)    | 13.5 (13)                   | 16.3 (8)                       |
| Ani s 1   | 12.5 (12)   | 2.7 (33)                    | 2.2 (29)                       |

\*  $p < 0.03$ ; \*\*  $p < 0.02$ , adjusted for multiple comparison (Bonferroni method). Molecules were ranked from more frequent to less frequent according to their occurrence in SS patients. In parentheses, the actual rank of each molecule in each group of patients is shown.

cells ( $2,899 \pm 4,328$  vs.  $975 \pm 1,215$  cells/ $\mu$ l,  $p < 0.05$ ) and lower number of CD4+CD26+ ( $130 \pm 183$  vs.  $367 \pm 462$  cells/ $\mu$ l,  $p < 0.05$ ) and CD4+CD49d+ ( $366 \pm 473$  vs.  $1,143 \pm 1,785$  cells/ $\mu$ l,  $p < 0.05$ ) in IgE+ patients (fig. 1). No other differences were observed comparing the other variables listed in table 1.

#### Allergenic Molecule-Specific IgE Production Is an Independent Marker of Prognostic Outcome in SS

Twenty-five subjects, still alive at the end of follow-up, had a lower prevalence of positive IgE reactivity (4 of 25 patients, 16%), when compared to patients with the poorest outcome (20 of 30, 66.6%) (Pearson  $\chi^2$ : 14.233,  $p < 0.001$ ). Such observations prompted us to verify if the clinical outcome could be related to specific IgE production.

Five-year survival probabilities for IgE production was  $82.2 \pm 8.3\%$  in IgE– patients, whilst IgE+ patients had an extremely low survival rate (25.5%, 5-year survival) as shown in figure 2a ( $p < 0.001$ ). Accordingly, the median survival times were 8.9 and 2.9 years for IgE– and IgE+, respectively ( $p < 0.001$ ).

We also estimate the survival for patients above and below different cutoffs for total IgE (8 kU/l being the most

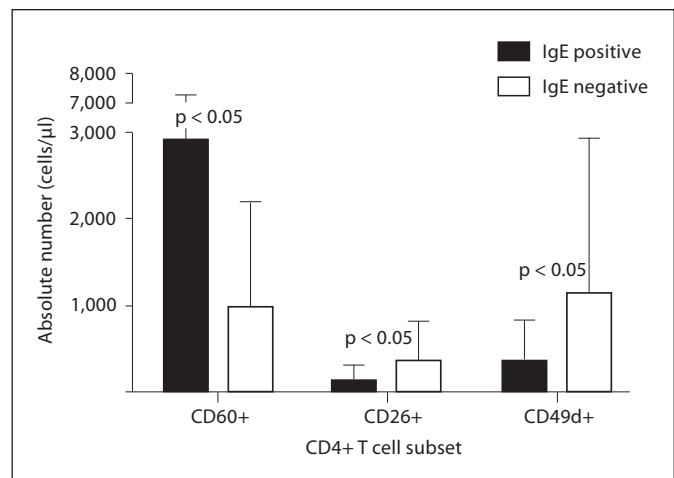


**Fig. 1.** CD4+ T cell subset absolute number comparison among IgE+ and IgE– patients with SS.

appropriate), and none resulted significant after a Kaplan-Meier analysis.

Taking advantage of our recent observation that up-regulation of CD60 and downregulation of CD49d on circulating CD4+ T cells are strongly associated with the poorest outcome in SS patients [37], we generated a new parameter combining these two variables with the ability to produce specific IgE. Three groups were identified by using this new combined parameter: the first including 9 IgE– patients with low levels of CD60 and normal expression of CD49d that were all still alive at the end of follow-up, the second (33 patients) with 1 of the 2 risk factors (median survival time 6.8 years) characterized by either IgE+ reactivity or upregulation of CD60 and downregulation of CD49d on circulating CD4+ T cells, and a third group represented by 14 patients having both risk factors with 2.1 years of median survival time.

Cox regression models were then applied to investigate whether, when simultaneously controlling for already well-known prognostic factors for SS (that is, CD4/CD8 ratio and lactate dehydrogenase serum levels), the IgE reactivity was still associated with survival. The final Cox model included gender, age, total lymphocyte count, TCR-V $\beta$  clone count, eosinophils, CD8+ T cell levels and IgE reactivity. Table 4 shows that specific IgE– remained significantly associated with survival.  $<150$  CD8 T cells/ $\mu$ l showed a hazard ratio of 2.1 with  $p = 0.073$ , very close to the usual conventional significance level. Specific IgE reactivity remained significantly associated with the outcome in all other multivariate models we tested.

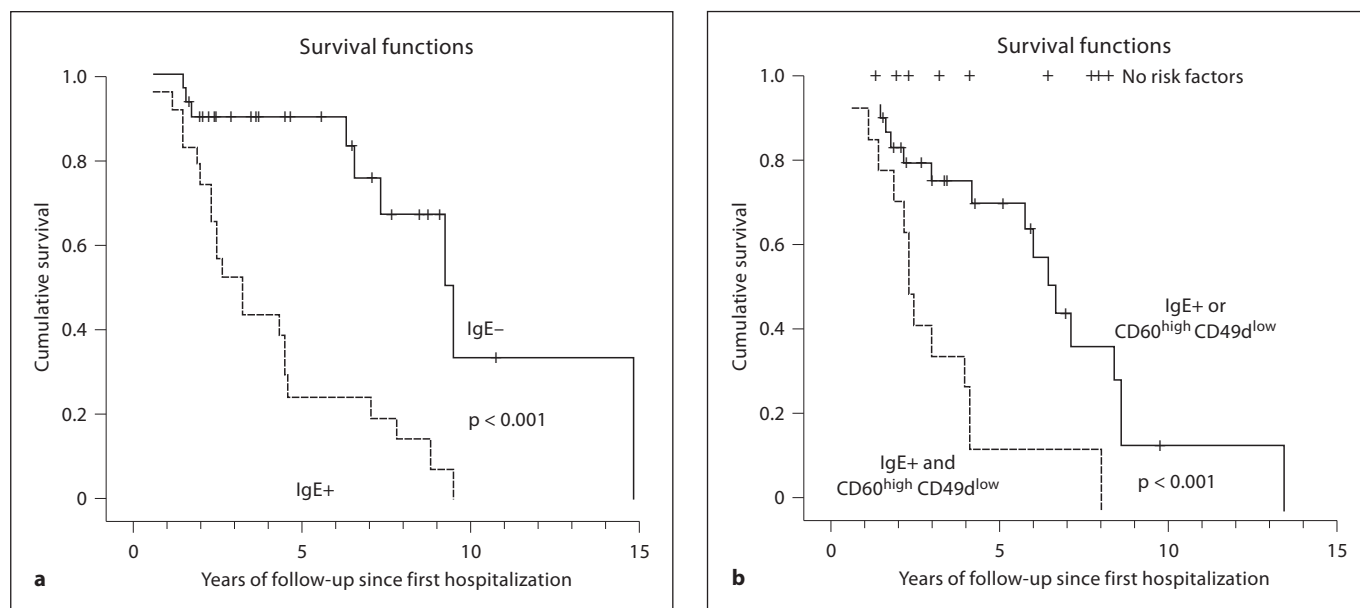


**Fig. 2.** Cumulative survival of 55 patients with SS, according to allergenic molecule-specific IgE production (a) or in association with other risk factors such as CD60 overexpression and CD49d downregulation (b).

**Table 4.** Multivariate analysis of prognostic factors for survival since first hospitalization for patients with SS

| Variable                                      | Level        | Hazard ratio | 95% CI   | p value   |
|-----------------------------------------------|--------------|--------------|----------|-----------|
| Total lymphocytes                             | $\geq 2,500$ | 0.6          | 0.2–2.3  | 0.383     |
| TCR-V $\beta$ clone, cells $\mu\text{l}^{-1}$ | $\geq 2,500$ | 0.6          | 0.2–2.0  | 0.438     |
| CD8+, cells $\mu\text{l}^{-1}$                | $< 150$      | 2.1          | 0.9–4.8  | 0.073     |
| Eos                                           | $> 350$      | 1.1          | 0.4–2.7  | 0.88      |
| IgE production                                |              | 7.1          | 2.6–19.4 | $< 0.001$ |

All hazard ratios are adjusted for gender and age at first hospital admission. Hazard ratios from Cox proportional model.

## Discussion

In this study we report the poorer outcome in SS to be predicted by the ability to produce specific IgE to at least one allergen among a comprehensive panel of molecules (fig. 2), whereas the measurement of total IgE levels does not allow, at least in our group of SS patients, the prediction of survival. It can be assumed that such detailed evaluation could never be performed in the past as SS patients cannot be skin tested because their skin disease is a contraindication, and because singleplex IgE testing cannot be as comprehensive as the microarray testing, otherwise

known as multiplexing. However, it is important to underline that the meaning of prognostic factor is not necessarily linked to that of causal factor: in fact, prognostic factors can be simply markers of an underlying process that actually modifies survival.

A number of epidemiological studies suggested a strong relationship between atopic conditions and hematopoietic cancers [18, 23, 48–51]. In these studies performed in allergic subjects, the presence of an increased risk of malignant lymphomas [50] or leukemia [52] has been described [51, 53]. By consequence, a chronic stimulation of the immune system could generate the occurrence of pro-oncogenic mutations in hematopoietic cells [35]. On the other hand, high IgE values occur also in other lymphomas (such as B cell lymphomas, also involving the skin), and often lymphoma is being diagnosed when the patients are investigated for hyper-IgE [54]. Herein we report a potential relationship between allergic reactivity and the worst outcome in a lymphoid tumor such as SS. As far as no differences in the severity of skin lesions between the two SS subsets (IgE+ vs. IgE– SS patients) have been reported by clinicians, we argue that the sensitization process is more the expression of a dysregulation of the immune system facilitating the IgE production in one subset rather than the other. The same mechanism could be involved in the different prognosis.

We report that the simultaneous presence of CD60 in circulating CD4+ T cell clone of IgE+ subjects is a stronger negative prognostic factor in SS. Conversely, the absence of risk factors such as specific IgE reactivity and overexpression of CD60 seems to identify a small group of patients with a very good prognosis (fig. 2b). With regard to this, several studies suggested a skewed Th2 cytokine profile in SS strongly related to the presence of a circulating malignant CD4+ T cell clone with Th2 features [7, 8, 14, 55–57]. It is widely known that IgE production is the result of Th2-skewed immune response, but also the presence of CD60 on T cells has been recently associated with functional Th2 profile [58, 59]. CD60 (a 9-0-acetylated form of GD3 ganglioside) is typically observed only in a small proportion of circulating human T cells, mainly expressed on the functional differentiated memory effector subset (CD45RO+) [59] and overexpressed in CD4+ SS cells [37, 47, 60–62]. Repeated stimulation of CD4+CD60+ memory T cells leads to the synthesis of Th2 cytokines such as IL-5, IL-4 and IL-10 [59, 63]; therefore, the simultaneous presence of IgE reactivity and CD60 expression could represent functional pathogenetic event in SS and this should be further investigated. By supervised class comparison analysis on gene expression profiles of purified T cells of IgE+ versus IgE– SS patients, we were able to identify a set of approximately 150 significant genes differentially expressed among the two classes. These genes mainly belong to Th1/Th2 differentiation, development, Fc  $\epsilon$  receptor I signaling, and cell adhesion molecules, and further validation is ongoing (data not shown).

Limited new data have been obtained by comparing the SS group of patients with control groups. Whether the minor differences in allergen IgE recognition (such as lower prevalence of Cup a 1 IgE+ subjects) could be further used as markers of severity requires a larger number of observations coming from the same environmental exposure. It is anyhow to be noted that the prevalence of Cup a 1 IgE+ subjects tends to increase in the last decade of life as reported by Scala et al. [38] and the SS patients studied are all in the 6th decade of life.

A further observation has been the presence of a specific IgE response to Sta a SEB in a small subset of SS patients. The skin of both AD and CTCL is often colonized by *S. aureus* strains generating a number of enterotoxins with superantigenic capacity, such as staphylococcal enterotoxins A, B, C, D, E and toxic shock toxin 1 [26, 27, 29, 32, 64–66]. All these superantigens can amplify the expression of skin-homing receptor (such as CLA) on T cells and induce the appearance of skewed T cell clones [26, 27, 32]. For example, toxic shock toxin 1 can selectively stim-

ulate TCR-V $\beta$ 2 bearing cells, staphylococcal enterotoxin C might bind both V $\beta$ 5.1 and V $\beta$ 13.1, staphylococcal enterotoxin B and staphylococcal enterotoxin C can excite V $\beta$ 17 and staphylococcal enterotoxin E can recognize V $\beta$ 5.1 [67–70]. A skewed usage of the TCR-V $\beta$  subfamilies 2, 5.1 and 13.1 in the circulating T cell clone of SS patients has been reported [37, 71]. A repeated superantigen stimulation of the TCR usually cause an activation-induced cell death [72], but the above described aberrant overexpression of CD60, which could play an antiapoptotic role in several lymphoid and myeloid cell types [73], and the well-known resistance to apoptosis of Th2 cells [74] could explain why a repetitive superantigen stimulation might eventually result in T cell oligoclonality [75].

In conclusion, in the present study we show that the measurement of specific IgE to allergenic molecules using a microarray-based system has a strong clinical and potentially pathogenetic impact on the assessment and prognosis of SS. Suggestions arise about the need of evaluating severe AD patients for both IgE immune response toward allergens using the multiplexing approach and periodically evaluating the CD60 expression along with the TCR-V $\beta$  repertoire. Furthermore, we should bear in mind that an IgE immune response could be directed to still not tested allergens, including autoallergens, not measured in our study.

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## References

- 1 Kim YH, Willemze R, Pimpinelli N, Whitaker S, Olsen EA, Ranki A, Dummer R, Hoppe RT: TNM classification system for primary cutaneous lymphomas other than mycosis fungoides and Sézary syndrome: a proposal of the International Society for Cutaneous Lymphomas (ISCL) and the Cutaneous Lymphoma Task Force of the European Organization of Research and Treatment of Cancer (EORTC). *Blood* 2007;110:479–484.
- 2 Hwang ST, Janik JE, Jaffe ES, Wilson WH: Mycosis fungoides and Sézary syndrome. *Lancet* 2008;371:945–957.

- 3 Girardi M, Heald PW, Wilson LD: The pathogenesis of mycosis fungoides. *N Engl J Med* 2004;350:1978–1988.
- 4 Jaffe ES: The 2008 WHO classification of lymphomas: implications for clinical practice and translational research. *Hematology Am Soc Hematol Educ Program* 2009;523–531.
- 5 Vardiman JW, Thiele J, Arber DA, Brunning RD, Borowitz MJ, Porwit A, Harris NL, Le Beau MM, Hellstrom-Lindberg E, Tefferi A, Bloomfield CD: The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. *Blood* 2009;114:937–951.
- 6 Papadavid E, Economidou J, Psarra A, Kapsimali V, Mantzana V, Antoniou C, Limas K, Stratigos A, Stavrianeas N, Avgerinou G, Katsambas A: The relevance of peripheral blood T-helper 1 and 2 cytokine pattern in the evaluation of patients with mycosis fungoides and Sézary syndrome. *Br J Dermatol* 2003;148:709–718.
- 7 Seo N, Tokura Y, Matsumoto K, Furukawa F, Takigawa M: Tumour-specific cytotoxic T lymphocyte activity in Th2-type Sézary syndrome: its enhancement by interferon-gamma (IFN- $\gamma$ ) and IL-12 and fluctuations in association with disease activity. *Clin Exp Immunol* 1998;112:403–409.
- 8 Saed G, Fivenson DP, Naidu Y, Nickoloff BJ: Mycosis fungoides exhibits a Th1-type cell-mediated cytokine profile whereas Sézary syndrome expresses a Th2-type profile. *J Invest Dermatol* 1994;103:29–33.
- 9 Tendler CL, Burton JD, Jaffe J, Danielpour D, Charley M, McCoy JP, Pittelkow MR, Waldmann TA: Abnormal cytokine expression in Sézary and adult T-cell leukemia cells correlates with the functional diversity between these T-cell malignancies. *Cancer Res* 1994;54:4430–4435.
- 10 Vowels BR, Cassin M, Vonderheid EC, Rook AH: Aberrant cytokine production by Sézary syndrome patients: cytokine secretion pattern resembles murine Th2 cells. *J Invest Dermatol* 1992;99:90–94.
- 11 Yagi H, Tokura Y, Furukawa F, Takigawa M: CD7-positive Sézary syndrome with a Th1 cytokine profile. *J Am Acad Dermatol* 1996;34:368–374.
- 12 Rogge JL, Hanifin JM: Immunodeficiencies in severe atopic dermatitis. Depressed chemotaxis and lymphocyte transformation. *Arch Dermatol* 1976;112:1391–1396.
- 13 Tancrede-Bohin E, Ionescu MA, de La SP, Dupuy A, Rivet J, Rybojad M, Dubertret L, Bachelez H, Lebbe C, Morel P: Prognostic value of blood eosinophilia in primary cutaneous T-cell lymphomas. *Arch Dermatol* 2004;140:1057–1061.
- 14 Dummer R, Geertsen R, Ludwig E, Niederer E, Burg G: Sézary syndrome, T-helper 2 cytokines and accessory factor-1 (AF-1). *Leuk Lymphoma* 1998;28:515–522.
- 15 Borish L, Dishuck J, Cox L, Mascali JJ, Williams J, Rosenwasser LJ: Sézary syndrome with elevated serum IgE and hypereosinophilia: role of dysregulated cytokine production. *J Allergy Clin Immunol* 1993;92:123–131.
- 16 Rook AH, Heald P: The immunopathogenesis of cutaneous T-cell lymphoma. *Hematol Oncol Clin North Am* 1995;9:997–1010.
- 17 Kural YB, Su O, Onsun N, Uras AR: Atopy, IgE and eosinophilic cationic protein concentration, specific IgE positivity, eosinophil count in cutaneous T cell lymphoma. *Int J Dermatol* 2010;49:390–395.
- 18 Hagstromer L, Ye W, Nyren O, Emtestam L: Incidence of cancer among patients with atopic dermatitis. *Arch Dermatol* 2005;141:1123–1127.
- 19 Rajka G, Winkelman RK: Atopic dermatitis and Sézary syndrome. *Arch Dermatol* 1984;120:83–84.
- 20 Van Haselen CW, Toonstra J, Preesman AH, Van Der Putte SC, Bruijnzeel-Koomen CA, van Vloten WA: Sézary syndrome in a young man with severe atopic dermatitis. *Br J Dermatol* 1999;140:704–707.
- 21 Abel EA, Nickoloff BJ, Shelby DM, Watson W, Wood GS: Tumor stage mycosis fungoides in a patient treated with long-term corticosteroids for asthma and atopic-like dermatitis. *J Dermatol Surg Oncol* 1986;12:1089–1093.
- 22 Meyer N, Mazereeuw-Hautier J, Launay F, Lamant L, Paul C: Cutaneous T cell lymphoma complicating severe atopic dermatitis. Is making a diagnosis the main challenge? *Dermatology* 2009;218:168–171.
- 23 Wang H, Diepgen TL: Atopic dermatitis and cancer risk. *Br J Dermatol* 2006;154:205–210.
- 24 Mehrany K, el-Azhary RA, Bouwhuis SA, Pittelkow MR: Cutaneous T-cell lymphoma and atopy: is there an association? *Br J Dermatol* 2003;149:1013–1017.
- 25 Zeller S, Glaser AG, Vilhelmsson M, Rhyner C, Cramer R: Immunoglobulin-E-mediated reactivity to self antigens: a controversial issue. *Int Arch Allergy Immunol* 2008;145:87–93.
- 26 Breuer K, Haussler S, Kapp A, Werfel T: *Staphylococcus aureus*: colonizing features and influence of an antibacterial treatment in adults with atopic dermatitis. *Br J Dermatol* 2002;147:55–61.
- 27 Bunikowski R, Mielke ME, Skarabis H, Worm M, Anagnostopoulos I, Kolde G, Wahn U, Renz H: Evidence for a disease-promoting effect of *Staphylococcus aureus*-derived exotoxins in atopic dermatitis. *J Allergy Clin Immunol* 2000;105:814–819.
- 28 Leung DY, Harbeck R, Bina P, Reiser RF, Yang E, Norris DA, Hanifin JM, Sampson HA: Presence of IgE antibodies to staphylococcal exotoxins on the skin of patients with atopic dermatitis. Evidence for a new group of allergens. *J Clin Invest* 1993;92:1374–1380.
- 29 Nomura I, Tanaka K, Tomita H, Katsunuma T, Ohya Y, Ikeda N, Takeda T, Saito H, Akasawa A: Evaluation of the staphylococcal exotoxins and their specific IgE in childhood atopic dermatitis. *J Allergy Clin Immunol* 1999;104:441–446.
- 30 Skov L, Olsen JV, Giorno R, Schlievert PM, Baadsgaard O, Leung DY: Application of staphylococcal enterotoxin B on normal and atopic skin induces up-regulation of T cells by a superantigen-mediated mechanism. *J Allergy Clin Immunol* 2000;105:820–826.
- 31 Torres MJ, Gonzalez FJ, Corzo JL, Giron MD, Carvajal MJ, Garcia V, Pinedo A, Martinez-Valverde A, Blanca M, Santamaria LF: Circulating CLA<sup>+</sup> lymphocytes from children with atopic dermatitis contain an increased percentage of cells bearing staphylococcal-related T-cell receptor variable segments. *Clin Exp Allergy* 1998;28:1264–1272.
- 32 Zollner TM, Wichelhaus TA, Hartung A, Von MC, Wagner TO, Brade V, Kaufmann R: Colonization with superantigen-producing *Staphylococcus aureus* is associated with increased severity of atopic dermatitis. *Clin Exp Allergy* 2000;30:994–1000.
- 33 Talpur R, Bassett R, Duvic M: Prevalence and treatment of *Staphylococcus aureus* colonization in patients with mycosis fungoides and Sézary syndrome. *Br J Dermatol* 2008;159:105–112.
- 34 Tokura Y, Yagi H, Ohshima A, Kurokawa S, Wakita H, Yokote R, Shirahama S, Furukawa F, Takigawa M: Cutaneous colonization with staphylococci influences the disease activity of Sézary syndrome: a potential role for bacterial superantigens. *Br J Dermatol* 1995;133:6–12.
- 35 Jackow CM, Cather JC, Hearne V, Asano AT, Musser JM, Duvic M: Association of erythrodermic cutaneous T-cell lymphoma, superantigen-positive *Staphylococcus aureus*, and oligoclonal T-cell receptor V $\beta$  gene expansion. *Blood* 1997;89:32–40.
- 36 Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J, Vardiman JW: WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, ed 4. Lyon, IARC Press, 2008.
- 37 Scala E, Abeni D, Pomponi D, Narducci MG, Lombardo GA, Mari A, Frontani M, Picchio MC, Pilla MA, Caprini E, Russo G: Predictive role of 9-O-acetylated ganglioside D3 (CD60) and  $\alpha$ 4 $\beta$ 1 (CD49d) expression in the survival of patients with Sézary syndrome. *Haematologica* 2010.
- 38 Scala E, Alessandri C, Bernardi ML, Ferrara R, Palazzo P, Pomponi D, Quarantino D, Rasi C, Zaffiro A, Zennaro D, Mari A: Cross-sectional survey on immunoglobulin E reactivity in 23,077 subjects using an allergenic molecule-based microarray detection system. *Clin Exp Allergy* 2010;40:911–921.
- 39 Deinhofer K, Sevcik H, Balic N, Harwanegg C, Hiller R, Rumpold H, Mueller MW, Spitzauer S: Microarrayed allergens for IgE profiling. *Methods* 2004;32:249–254.

- 40 Hiller R, Laffer S, Harwanegg C, Huber M, Schmidt WM, Twardosz A, Barletta B, Becker WM, Blaser K, Breiteneder H, Chapman M, Cramer R, Duchene M, Ferreira F, Fiebig H, Hoffmann-Sommergruber K, King TP, Kleber-Janke T, Kurup VP, Lehrer SB, Lidholm J, Muller U, Pini C, Reese G, Scheiner O, Scheynius A, Shen HD, Spitzauer S, Suck R, Swoboda I, Thomas W, Tinghino R, Van Hage-Hamsten M, Virtanen T, Kraft D, Muller MW, Valenta R: Microarrayed allergen molecules: diagnostic gatekeepers for allergy treatment. *FASEB J* 2002;16:414–416.
- 41 Harwanegg C, Laffer S, Hiller R, Mueller MW, Kraft D, Spitzauer S, Valenta R: Microarrayed recombinant allergens for diagnosis of allergy. *Clin Exp Allergy* 2003;33:7–13.
- 42 Kaplan EL, Meier P: Nonparametric estimation from incomplete observations. *J Am Stat Assoc* 1958;53:457–481.
- 43 Sackett DL, Haynes RB, Guyatt GH, Tugwell T: *Clinical epidemiology: a basic science for clinical medicine*. Boston, MA, USA, 1991.
- 44 Cox RD: *Regression models and life-tables*. *J R Stat Soc B* 1972;34:187–220.
- 45 Abeni D, Frontani M, Sampogna F, Sera F, Bolli S, Corona R, Baliva G, Russo G: Circulating CD8+ lymphocytes, white blood cells, and survival in patients with mycosis fungoides. *Br J Dermatol* 2005;153:324–330.
- 46 Narducci MG, Scala E, Bresin A, Caprini E, Picchio MC, Remotti D, Ragone G, Nasorri F, Frontani M, Arcelli D, Volinia S, Lombardo GA, Baliva G, Napolitano M, Russo G: Skin homing of Sezary cells involves SDF-1-CXCR4 signaling and down-regulation of CD26/dipeptidylpeptidase IV. *Blood* 2006;107:1108–1115.
- 47 Scala E, Narducci MG, Amerio P, Baliva G, Simoni R, Giovannetti A, Silvestri L, Puddu P, De Pita O, Russo G: T cell receptor-V $\beta$  analysis identifies a dominant CD60+ CD26– CD49d– T cell clone in the peripheral blood of Sézary syndrome patients. *J Invest Dermatol* 2002;119:193–196.
- 48 Arana A, Wentworth CE, Fernandez-Vidaurre C, Schlienger RG, Conde E, Arellano F: Incidence of cancer in the general population and in patients with or without atopic dermatitis in the UK. *Br J Dermatol* 2010.
- 49 Zhang Y, Holford TR, Leaderer B, Zahm SH, Boyle P, Morton LM, Zhang B, Zou K, Flynn S, Tallini G, Owens PH, Zheng T: Prior medical conditions and medication use and risk of non-Hodgkin lymphoma in Connecticut United States women. *Cancer Causes Control* 2004;15:419–428.
- 50 Eriksson NE, Holmen A, Hogstedt B, Mikoczy Z, Hagmar L: A prospective study of cancer incidence in a cohort examined for allergy. *Allergy* 1995;50:718–722.
- 51 Soderberg KC, Hagmar L, Schwartzbaum J, Feychting M: Allergic conditions and risk of hematological malignancies in adults: a cohort study. *BMC Public Health* 2004;4:51.
- 52 Talbot-Smith A, Fritsch L, Divitini ML, Mallon DF, Knuiman MW: Allergy, atopy, and cancer: a prospective study of the 1981 Busselton cohort. *Am J Epidemiol* 2003;157:606–612.
- 53 Gurney KA, Cartwright RA: Increasing incidence and descriptive epidemiology of extranodal non-Hodgkin lymphoma in parts of England and Wales. *Hematol J* 2002;3:95–104.
- 54 Ellis AK, Wasserman S: Hodgkin's lymphoma presenting with markedly elevated IgE: a case report. *Allergy Asthma Clin Immunol* 2009;5:12.
- 55 Dummer R, Heald PW, Nestle FO, Ludwig E, Laine E, Hemmi S, Burg G: Sézary syndrome T-cell clones display T-helper 2 cytokines and express the accessory factor-1 (interferon- $\gamma$  receptor  $\beta$ -chain). *Blood* 1996;88:1383–1389.
- 56 Doherty SD, Ni X, Doherty CB, Jones D, Zhao X, Owen LB, Duvic M: Abnormal expression of interleukin-23 in mycosis fungoides/Sézary syndrome lesions. *Arch Dermatol Res* 2006;298:353–356.
- 57 Kim EJ, Hess S, Richardson SK, Newton S, Showe LC, Benoit BM, Ubriani R, Vittorio CC, Junkins-Hopkins JM, Wysocka M, Rook AH: Immunopathogenesis and therapy of cutaneous T cell lymphoma. *J Clin Invest* 2005;115:798–812.
- 58 Smith-Norowitz TA, Norowitz KB, Silverberg JJ, Chice S, Shah V, Stanek A, Walker D, Brennan JP, Durkin HG, Bluth MH: CD8+CD60+ T cells, cells expressing epsilon specific mRNA and Th1/Th2 cytokines in cord blood and at 7 months of age. *Scand J Immunol* 2008;68:526–533.
- 59 Wada T, Seki H, Konno A, Ohta K, Nunogami K, Kaneda H, Kasahara Y, Yachie A, Koizumi S, Taniguchi N, Miyawaki: Developmental changes and functional properties of human memory T cell subpopulations defined by CD60 expression. *Cell Immunol* 1998;187:117–123.
- 60 Hansen ER, Vejlsgaard GL, Cooper KD, Heidenheim M, Larsen JK, Ho VC, Ross CW, Fox DA, Thomsen K, Baadsgaard O: Leukemic T cells from patients with cutaneous T-cell lymphoma demonstrate enhanced activation through CDw60, CD2, and CD28 relative to activation through the T-cell antigen receptor complex. *J Invest Dermatol* 1993;100:667–673.
- 61 Scala E, Russo G, Cadoni S, Narducci MG, Girardelli CR, De PO, Puddu P: Skewed expression of activation, differentiation and homing-related antigens in circulating cells from patients with cutaneous T cell lymphoma associated with CD7– T helper lymphocytes expansion. *J Invest Dermatol* 1999;113:622–627.
- 62 Jones D, Dang NH, Duvic M, Washington LT, Huh YO: Absence of CD26 expression is a useful marker for diagnosis of T-cell lymphoma in peripheral blood. *Am J Clin Pathol* 2001;115:885–892.
- 63 Semnani RT, Nutman TB, Hochman P, Shaw S, van Seventer GA: Costimulation by purified intercellular adhesion molecule 1 and lymphocyte function-associated antigen 3 induces distinct proliferation, cytokine and cell surface antigen profiles in human 'naive' and 'memory' CD4+ T cells. *J Exp Med* 1994;180:2125–2135.
- 64 Mempel M, Lina G, Hojka M, Schnopp C, Seidl HP, Schafer T, Ring J, Vandenesh F, Abeck D: High prevalence of superantigens associated with the EGC locus in *Staphylococcus aureus* isolates from patients with atopic eczema. *Eur J Clin Microbiol Infect Dis* 2003;22:306–309.
- 65 Akiyama H, Toi Y, Kanzaki H, Tada J, Arata J: Prevalence of producers of enterotoxins and toxic shock syndrome toxin-1 among *Staphylococcus aureus* strains isolated from atopic dermatitis lesions. *Arch Dermatol Res* 1996;288:418–420.
- 66 McFadden JP, Noble WC, Camp RD: Superantigenic exotoxin-secreting potential of staphylococci isolated from atopic eczematous skin. *Br J Dermatol* 1993;128:631–632.
- 67 Kotzin BL, Leung DY, Kappler J, Marrack P: Superantigens and their potential role in human disease. *Adv Immunol* 1993;54:99–166.
- 68 Leung DY, Gately M, Trumble A, Ferguson-Darnell B, Schlievert PM, Picker LJ: Bacterial superantigens induce T cell expression of the skin-selective homing receptor, the cutaneous lymphocyte-associated antigen, via stimulation of interleukin 12 production. *J Exp Med* 1995;181:747–753.
- 69 Proft T, Fraser JD: Bacterial superantigens. *Clin Exp Immunol* 2003;133:299–306.
- 70 Davison S, Allen M, Vaughan R, Barker J: Staphylococcal toxin-induced T cell proliferation in atopic eczema correlates with increased use of superantigen-reactive V $\beta$ -chains in cutaneous lymphocyte-associated antigen (CLA)-positive lymphocytes. *Clin Exp Immunol* 2000;121:181–186.
- 71 Vonderheid EC, Boselli CM, Conroy M, Casaus L, Espinoza LC, Venkataramani P, Bigler RD, Hou JS: Evidence for restricted V $\beta$  usage in the leukemic phase of cutaneous T cell lymphoma. *J Invest Dermatol* 2005;124:651–661.
- 72 Weber AK, Wahn U, Renz H: Superantigen-induced T cell death by apoptosis: analysis on a single cell level and effect of IFN- $\gamma$  and IL-4 treatment. *Int Arch Allergy Immunol* 2000;121:215–223.
- 73 Knip E, Knip E, Ozkucur N, Barz S, Bachmann M, Malisan F, Testi R, Rieber EP: 9-O-acetyl GD3 protects tumor cells from apoptosis. *Int J Cancer* 2006;119:67–73.
- 74 Akdis M, Trautmann A, Klunker S, Daigle I, Kucuksezir UC, Deglmann W, Disch R, Blaser K, Akdis CA: T helper (Th) 2 predominance in atopic diseases is due to preferential apoptosis of circulating memory/effector Th1 cells. *FASEB J* 2003;17:1026–1035.
- 75 Kim KS, Jacob N, Stohl W: In vitro and in vivo T cell oligoclonality following chronic stimulation with staphylococcal superantigens. *Clin Immunol* 2003;108:182–189.