

receives honoraria from GlaxoSmithKline; is a board member of the APFED; holds equity in Immune Design Corporation; is a founder of ImmViz; can receive royalties from Teva/Cephalon; receives royalties from the Mayo Foundation; and is the spouse of K. M. Leiferman. K. A. Peterson has received financial support from the 2010 Castell Esophageal Grant, the Utah Governor's Office of Economic Development, and the APFED; is employed by the University of Utah; and has a patent filed through the University of Utah on the radiolabeled heparin presented in this study. L. F. Pease has received grants from the National Science Foundation, the NIH, the Utah Governor's Office of Economic Development, and the APFED; is employed by the University of Utah; and has a patent filed through the University of Utah on the radiolabeled heparin presented in this study. F. C. Clayton declares no relevant conflicts of interest.

REFERENCES

- Gonsalves N, Policarpio-Nicolas M, Zhang Q, Rao MS, Hirani I. Histopathologic variability and endoscopic correlates in adults with eosinophilic esophagitis. *Gastrointest Endosc* 2006;64:313-9.
- Saffari H, Peterson KA, Fang JC, Teman C, Gleich GJ, Pease LF. Patchy eosinophil distributions in an esophagectomy specimen from a patient with eosinophilic esophagitis: implications for endoscopic biopsy. *J Allergy Clin Immunol* 2012;130:798-800.
- Swaminathan GJ, Myszka DG, Katsamba PS, Ohunki LE, Gleich GJ, Acharya KR. Eosinophil-granule major basic protein, a C-type lectin, binds heparin. *Biochemistry* 2006;44:14152-8.
- Kephart GM, Alexander JA, Arora AS, Romero Y, Smyrk TC, Talley NJ, et al. Marked deposition of eosinophil-derived neurotoxin in adult patients with eosinophilic esophagitis. *Am J Gastroenterol* 2010;105:298-307.
- Talley NJ, Kephart GM, McGovern TW, Carpenter HA, Gleich GJ. Deposition of eosinophil granule major basic protein in eosinophilic gastroenteritis and celiac disease. *Gastroenterology* 1992;103:137-45.
- Hirash J, Warkentin TE, Shaughnessy SG, Anand SS, Halperin JL, Raschke R, et al. Heparin and low-molecular-weight heparin: mechanisms of action, pharmacokinetics, dosing, monitoring, efficacy, and safety. *Chest* 2001;119:64S-94S.
- Laforest MD, Colas-Linhart N, Guiraud-Vitaux F, Bok B, Bara L, Samama M, et al. Pharmacokinetics and biodistribution of technetium 99m labelled standard heparin and a low molecular weight heparin (enoxaparin) after intravenous injection in normal volunteers. *Br J Haematol* 1991;77:201-8.
- Kulkarni PV, Parkey RW, Wilson JE III, Lewis SE, Buja LM, Bonte FJ, et al. Modified technetium-99m heparin for the imaging of acute experimental myocardial infarcts. *J Nucl Med* 1980;21:117-21.
- Johnson DA, Barton GJ. Mast cell tryptases: examination of unusual characteristics by multiple sequence alignment and molecular modeling. *Protein Sci* 1992;1:370-7.
- Perez GL, Peters MS, Reda AM, Butterfield JH, Peterson EA, Leiferman KM. Mast cells, neutrophils, and eosinophils in prurigo nodularis. *Arch Dermatol* 1993;129:861-5.

Available online July 26, 2013.

<http://dx.doi.org/10.1016/j.jaci.2013.06.009>

CD39: A new surface marker of mouse regulatory $\gamma\delta$ T cells

To the Editor:

$\gamma\delta$ T cells have many characteristic features and functions. Activated $\gamma\delta$ T cells release cytokines, such as IFN- γ , IL-10, and IL-17.¹ A subset of $\gamma\delta$ T cells that express CD27 produce IFN- γ , whereas IL-17 production is restricted to CD27⁻ $\gamma\delta$ T cells.² In addition, a CD25⁺ CD122⁻ $\gamma\delta$ T subset produces IL-17.³ However, a subset of $\gamma\delta$ T cells can suppress T-cell and dendritic cell (DC) functions.⁴ Consistently, adoptively transferred $\gamma\delta$ T cells regulated graft-versus-host reactivity in mice.⁵ Therefore, the concept of regulatory $\gamma\delta$ T cells exists; however, it has been difficult to evaluate the roles of regulatory $\gamma\delta$ T cells because a marker of regulatory $\gamma\delta$ T cells remains unclarified.

In this study, we sought to identify a marker of these regulatory $\gamma\delta$ T cells. Regulatory T (Treg) cells are well established as immunosuppressants. They express several markers, including CD25, forkhead box P3 (Foxp3), cytotoxic T-lymphocyte antigen 4 (CTLA-4), and CD39.⁶ We first analyzed the expression of these markers on $\gamma\delta$ T cells in axillary and inguinal lymph nodes with mouse by using the FACS Fortessa flow cytometric system (BD Bioscience, San Diego, Calif). We found that Thy1.2⁺ $\gamma\delta$ TCR⁺

$\gamma\delta$ T cells had a population of CD39⁺ (all antibodies for flow cytometry from eBioscience, San Diego, Calif) (Fig 1, A). CD39 is known for ectoenzyme that removes the nucleotide by hydrolytic cleavage and exhibits immunomodulatory influences on its proximal environment.⁶ We next analyzed other markers of $\gamma\delta$ T cells and identified that CD39⁺ $\gamma\delta$ T cells expressed CD25 but not CD27 or CD122 (Fig 1, A). In addition, CD39⁺ $\gamma\delta$ T cells did not express programmed death-1 (Fig 1, A), Foxp3 (Fig 1, B), or CTLA-4 (data not shown). Because $\gamma\delta$ T cells did not express Foxp3 or CTLA-4, we focused on the CD25⁺ and CD39⁺ $\gamma\delta$ T-cell subset for further study.

Next, we examined by using a T-cell proliferation assay whether CD25⁺ and CD39⁺ $\gamma\delta$ T cells have an immunosuppressive activity like Treg cells. CD4⁺ CD25⁻ T cells and DCs were sorted from splenocytes by using auto-MACS (Miltenyi Biotech, Bergisch Gladbach, Germany). CD39⁺ and CD39⁻ $\gamma\delta$ T cells were sorted from axillary and inguinal lymph nodes by using a FACS Aria II cell sorter (BD Bioscience). Then, CD4⁺ CD25⁻ T cells (5×10^4 cells/well) were cultured with DCs (2×10^4 cells/well) with or without CD39⁺ $\gamma\delta$ T cells ($5, 2.5, 1 \times 10^4$ cells/well), CD39⁻ $\gamma\delta$ T cells ($5, 2.5, 1 \times 10^4$ cells/well), or Treg cells (1×10^4 cells/well) in a 96-well plate. For the last 24 hours of incubation, the cells were pulsed with 0.5 μ Ci ³H-thymidine to measure cell proliferation. In contrast to CD39⁻ $\gamma\delta$ T cells, CD39⁺ $\gamma\delta$ T cells suppressed T-cell proliferation in a dose-dependent manner (Fig 1, C). Because CD39⁻ $\gamma\delta$ T cells included a CD25⁺ $\gamma\delta$ T-cell subset (Fig 1, A), we considered that CD39 is a candidate of a surface marker of regulatory $\gamma\delta$ T cells. Intriguingly, CD39⁺ $\gamma\delta$ T cells did not suppress T-cell proliferation in the absence of DCs using a plate coated with 1 μ g/mL of anti-CD3 antibody, although Treg cells suppressed T-cell proliferation even in the absence of DCs (Fig 1, D). These results suggest that the suppressive activity of CD39⁺ $\gamma\delta$ T cells depend on the presence of DCs.

To identify what mediates the suppressive activity, we performed quantitative RT-PCR analysis. Total RNA from cells was isolated with an RNeasy Mini Kit (QIAGEN, Hilden, Germany). Quantitative RT-PCR with a LightCycler real-time PCR apparatus was performed by using SYBR Green I (Roche, Basel, Switzerland). The results were normalized to the glyceraldehyde-3-phosphate dehydrogenase mRNA level. Because $\gamma\delta$ T cells produce IL-10, which acts as an immunosuppressive,^{1,7} we evaluated the IL-10 mRNA levels. The mRNA levels of IL-10 in CD39⁺ $\gamma\delta$ T cells were significantly higher than in CD39⁻ $\gamma\delta$ T cells (Fig 1, E). However, the mRNA levels of IFN- γ in CD39⁺ $\gamma\delta$ T cells were significantly lower than in CD39⁻ $\gamma\delta$ T cells. To address whether IL-10 in CD39⁺ $\gamma\delta$ T cells is the main suppressive cytokine of regulatory $\gamma\delta$ T cells, we examined a T-cell proliferation assay using IL-10 knockout mice (kindly provided by Dr K. Suto, Tokyo Medical University). Because CD39⁺ $\gamma\delta$ T cells sorted from IL-10 knockout mice did not suppress T-cell proliferation (Fig 1, F), we concluded that IL-10 was the pivotal suppressive cytokine of CD39⁺ $\gamma\delta$ T cells.

In addition, we successfully induced CD39⁺ $\gamma\delta$ T cells from CD39⁻ $\gamma\delta$ T cells under a condition of anti-CD3 stimulation and IL-2 for 10 days, which confirmed that almost all $\gamma\delta$ T cells expressed CD39 (Fig 2, A). We next analyzed the surface marker of *in vitro*-induced CD39⁺ $\gamma\delta$ T cells. CD39⁺ $\gamma\delta$ T cells expressed CD27 and CD122 but did not express CD4, CD8, or Foxp3 (Fig 2, B). Therefore, naive CD39⁺ $\gamma\delta$ T cells and *in vitro*-induced CD39⁺ $\gamma\delta$ T cells showed a different expression

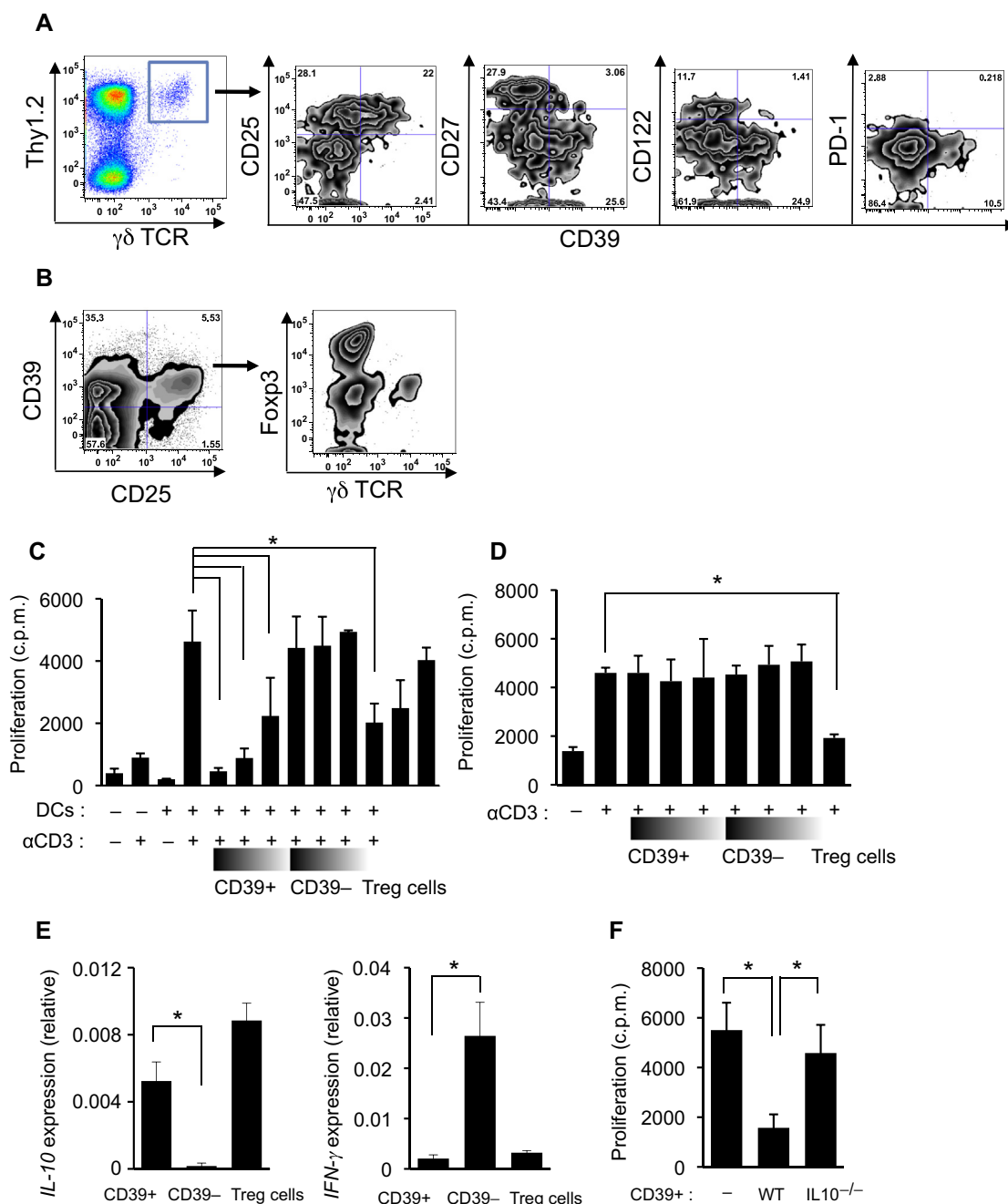


FIG 1. The expression and suppressive activity of CD39⁺ $\gamma\delta$ T cells. **A**, The expression of CD39, CD27, CD122, CD25, and programmed death-1 (PD-1) on Thy1.2⁺ $\gamma\delta$ TCR⁺ $\gamma\delta$ T cells from axillary and inguinal lymph nodes. **B**, The expression of Foxp3 on CD39⁺ $\gamma\delta$ T cells. **C**, The proliferative activity with CD39⁻, CD39⁺ $\gamma\delta$ T cells, or Treg cells. **D**, The proliferative activity in the absence of DCs. **E**, The mRNA level of IL-10 and IFN- γ of CD39⁺, CD39⁻ $\gamma\delta$ T, and Treg cells. **F**, The proliferative activity with CD39⁺ $\gamma\delta$ T cells from wild-type or IL-10 knockout (IL10^{-/-}) mice. All data are presented as the mean $\pm$ SD (n = 5) and are representative of 3 experiments. *P < .05 (Wilcoxon signed-rank test) versus corresponding groups.

of the same markers. Then, we addressed whether *in vitro*-induced CD39⁺ $\gamma\delta$ T cells have a suppressive activity by using the proliferation assay. As naive CD39⁺ $\gamma\delta$ T cells have a suppressive activity, as demonstrated in Fig 1, C, *in vitro*-induced CD39⁺ $\gamma\delta$ T cells have a strong suppressive activity *in vitro* (Fig 2, C). Finally, we examined an *in vivo* immunosuppression assay by using a contact hypersensitivity reaction model, which

is a prototype of skin allergic reaction.⁸ We sensitized mice with 25 μ L of 0.5% dinitrofluorobenzene (Nacalai Tesque, Kyoto, Japan) in acetone/olive oil (4:1) on shaved abdominal skin. Five days later, a total of 4×10^3 cells of isolated CD4⁺ T cells or the *in vitro*-induced CD39⁺ $\gamma\delta$ T cells in 20 μ L PBS were subcutaneously injected into the ventral surface of each ear. Ear thickness was measured before and at the indicated

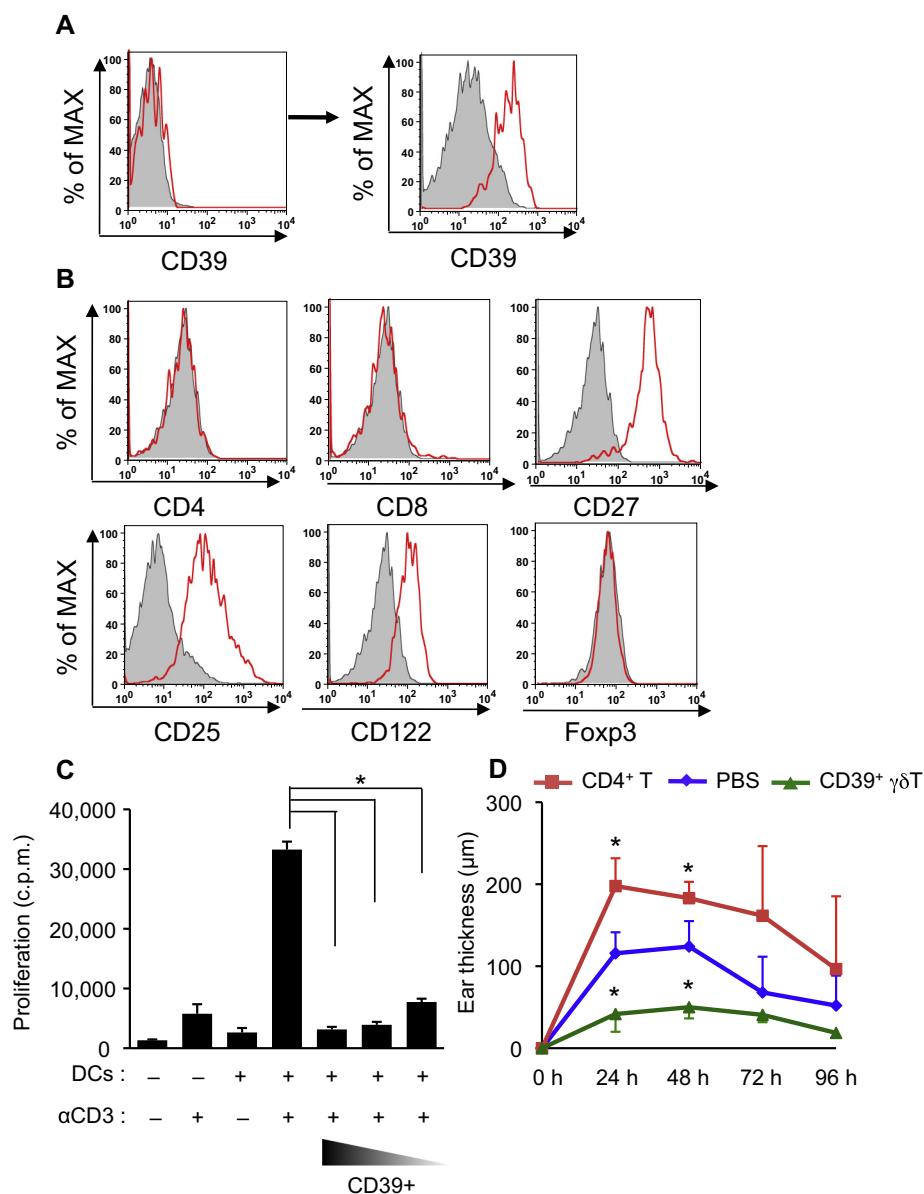


FIG 2. The expression and suppressive activity of *in vitro*-induced CD39⁺ $\gamma\delta$ T cells. **A**, The expression of CD39 on $\gamma\delta$ T cells before (left panel) and after (right panel) *in vitro* stimulation. **B**, The expression of CD4, CD8, CD27, CD122, Foxp3, and CD25 on CD39⁺ $\gamma\delta$ T cells. **C**, The proliferative activity with *in vitro*-induced CD39⁺ $\gamma\delta$ T cells. **D**, The time course of the contact hypersensitivity reaction with adoptive cell transfer of CD4⁺ or CD39⁺ $\gamma\delta$ T cells. All data are presented as the mean $\pm$ SD (n = 5) and are representative of 3 experiments. **P* < .05 (Wilcoxon signed-rank test) versus corresponding groups.

time points after elicitation with 20 μ L of 0.3% dinitrofluorobenzene. Consistent with the *in vitro* findings, the injection of *in vitro*-induced CD39⁺ $\gamma\delta$ T cells significantly suppressed ear swelling, whereas the injection of CD4⁺ T cells rather promoted ear swelling (Fig 2, D).

In conclusion, we have identified a candidate of a new surface marker of mouse regulatory $\gamma\delta$ T cells, which have immunosuppressive activity possibly via IL-10. In addition, we demonstrated that CD39⁺ $\gamma\delta$ T cells were induced from CD39⁺ $\gamma\delta$ T cells *in vitro* and have a strong suppressive activity both *in vitro* and *in vivo*. From a clinical perspective, our results may prove relevant to adoptive immunotherapy of regulatory $\gamma\delta$ T cells to allergic skin reactions.

We thank Ms Hiromi Doi for technical assistance.

Atsushi Otsuka, MD, PhD
Sho Hanakawa, MS
Yoshiki Miyachi, MD, PhD
Kenji Kabashima, MD, PhD

From the Department of Dermatology, Kyoto University Graduate School of Medicine, Kyoto, Japan. E-mail: kaba@kuhp.kyoto-u.ac.jp.

This work was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology and the Ministry of Health, Labor and Welfare of Japan. No additional external funding was received for this study.

Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest.

REFERENCES

1. Vantourout P, Hayday A. Six-of-the-best: unique contributions of gammadelta T cells to immunology. *Nat Rev Immunol* 2013;13:88-100.
2. Ribot JC, deBarros A, Pang DJ, Neves JF, Peperzak V, Roberts SJ, et al. CD27 is a thymic determinant of the balance between interferon-gamma- and interleukin 17-producing gammadelta T cell subsets. *Nat Immunol* 2009;10:427-36.
3. Shibata K, Yamada H, Nakamura R, Sun X, Itsumi M, Yoshikai Y. Identification of CD25+ gamma delta T cells as fetal thymus-derived naturally occurring IL-17 producers. *J Immunol* 2008;181:5940-7.
4. Peng G, Wang HY, Peng W, Kiniwa Y, Seo KH, Wang RF. Tumor-infiltrating gammadelta T cells suppress T and dendritic cell function via mechanisms controlled by a unique toll-like receptor signaling pathway. *Immunity* 2007;27:334-48.
5. Drobyski WR, Vodanovic-Jankovic S, Klein J. Adoptively transferred gamma delta T cells indirectly regulate murine graft-versus-host reactivity following donor leukocyte infusion therapy in mice. *J Immunol* 2000;165:1634-40.
6. Borsellino G, Kleinschewitz M, Di Mitri D, Sternjak A, Diamantini A, Giometto R, et al. Expression of ectonucleotidase CD39 by Foxp3+ Treg cells: hydrolysis of extracellular ATP and immune suppression. *Blood* 2007;110:1225-32.
7. Hao J, Wu X, Xia S, Li Z, Wen T, Zhao N, et al. Current progress in gammadelta T-cell biology. *Cell Mol Immunol* 2010;7:409-13.
8. Honda T, Egawa G, Grabbe S, Kabashima K. Update of immune events in the murine contact hypersensitivity model: toward the understanding of allergic contact dermatitis. *J Invest Dermatol* 2013;133:303-15.

Available online July 16, 2013.
<http://dx.doi.org/10.1016/j.jaci.2013.05.037>

Anhidrotic ectodermal dysplasia: A new mutation

To the Editor:

Hyper-IgM syndrome is a genetically heterogeneous clinical condition, characterized by high/normal levels of IgM with low/absent levels of IgA, IgG, and IgE, indicating a defect in class-switch recombination. Hyper-IgM immunodeficiency may be associated with the anhidrotic ectodermal dysplasia with immunodeficiency (EDA-ID), caused by mutations in the gene encoding either nuclear factor kappa B (NF- κ B) essential modulator (*NEMO*)/I κ B kinase γ (*IKK γ*) (X-linked-EDA-ID)

or the inhibitor of NF- κ B (*I κ B*) regulated by the IKK complex, which includes I κ B α , I κ B β , and I κ B ϵ (autosomal-EDA-ID).^{1,2} We describe an infant with EDA-ID caused by a new mutation of *I κ B α* , not yet reported in the literature.

A 5-month-old female infant born to unrelated parents, sparse-haired and with evident superficial cutaneous *venous reticulum* on her dry and thin skin, was hospitalized for bilateral bronchopneumonia, fever, and suspected septicemia resistant to antibiotic and steroid therapy. She presented hypogammaglobulinemia (IgG 0.3 g/L, IgA <0.05 g/L) with increasing value of IgM (7 g/L), absence of allohemagglutinin titers, and no antibody response to tetanus toxoid and *Haemophilus influenzae* (Table I). So, substitutive therapy with intravenous immunoglobulins (400-800 mg/kg/month) was administered with little effect: in the period between age 6 and 12 months, she developed severe respiratory failure, chronic diarrhea, vomiting, loss of appetite and fever, with increased inflammation indexes, followed by 2 episodes of sepsis by opportunistic organisms, respectively, *Stenotrophomonas maltophilia* and *Candida albicans*, diffuse impetigo, and osteomyelitis of skull and limbs. Three consecutive sweat tests failed for hypohidrosis, while the other metabolic investigations all resulted as normal. The sudden worsening of clinical conditions stopped us from carrying out the planned stem cell transplant, and the patient was placed in a laminar-flow room. Despite this measure, the infant first developed wide oral candidiasis involving the esophagus, then sepsis by *Klebsiella pneumoniae*, and finally sepsis by *Candida parapsilosis*, complicated with fatal disseminated intravascular coagulation. During the hospitalization, we performed immunologic and genetic studies.

At the age of 5 months, T cells were present at the expected frequency (57% of the cells in the lymphocyte gate) and the ratio CD4/CD8 was normal. The frequency of CD45ROpos memory B cells was low-normal for the age (5% of T lymphocytes). T-cell proliferation to anti-CD3-CD28, evaluated

TABLE I. Immunologic characteristics of the patient

Age at evaluation	Lymphocyte flow cytometry (absolute values)					Mitogen stimulation	Immunoglobulins (g/L)			Vaccine titers	
	CD3	CD4	CD8	CD16/56	CD19/20		IgG	IgA	IgM	Tetanus (IU/mL)	Hib (mg/L)
6 mo	15008	7616	5824	224	7168	Low response of B cells stimulated with CpG (2 samples)	0.3	0.11	3.04		
7 mo							0.3	0.05	7		
8 mo*						Normal response of T cells with anti-CD3+CD28+; no alteration of proliferative response after PMA and PHA (2 samples)	2.1	0.6	2.33		
10 mo*	14268	9184	5084	328	1476		5.2	0.09	1.77	0.05	0.1
12 mo						Low response of B cells stimulated with CpG (2 samples)	4.4	0.13	1.02		
13 mo	21080	12896	7936	992	2480		5.1	0.23	0.95		
14 mo*						Normal response of T cell with anti-CD3+CD28; no significant alteration of proliferative response after PMA and PHA (2 samples)	10.9	0.33	1.9		

Hib, *Haemophilus influenzae b*; PMA, phorbol 12-myristate 13-acetate plus ionomycin.

*After intravenous immunoglobulins (IVIGs).