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Original Article

Induction of IgE synthesis by genetically modified CD8+ T cells of a patient with adenosine deaminase deficiency

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Abstract

We have previously reported that an adenosine deaminase (ADA)-deficient patient treated with T cell-directed gene therapy had an increase in serum IgE levels, despite a marked inversion of the CD4/CD8 ratio. In the present report, we have analyzed the phenotypic and functional profiles of the patient's lymphocytes obtained during the clinical trial. In peripheral blood mononuclear cells (PBMC) that were freshly prepared from the patient, both $CD4^+$ and $CD8^+$ T cell subsets were negative for CD40 ligand (CD40L; CD154) and Fas ligand (FasL; CD95L), while $CD20^+$ B cells constitutively expressed CD40 and HLA-DR and were negative for CD80, CD86 and Fas (CD95). The expression of CD23 was detected on the majority of $CD20^+$ B cells and expression was upregulated by interleukin (IL)-4. Furthermore, the patient's PBMC, which already expressed both germline and mature Cε transcripts *in vivo*, spontaneously secreted IgE and responded to IL-4 with increased IgE production during *in vitro* culture. When stimulated with anti-CD3ε monoclonal antibody (mAb), $CD8^+$ T cells from gene-transduced T cells displayed high production of interferon (IFN)-γ, low production of IL-4 and IL-13 and comparable levels of CD40L and FasL expression; however, lined $CD8^+$ T cells from circulating T cells expressing the transgene produced IL-4 and IL-13 together with smaller amounts of IFN-γ and preferably expressed CD40L rather than FasL. Two such $CD8^+$ T cells, in conjunction with the presence of IL-4, supported CD40L-mediated B cell proliferation and IgE production after stimulation and fixation. These results indicate that ADA-deficient B cells are functionally mature and that gene-transduced $CD8^+$ T cells and lined $CD8^+$ T cells containing the transgene exhibit T helper 0- and T cytotoxic (c) 2-like phenotypes, respectively. Our data also suggest that immuno-logic reconstitution with genetically modified $CD8^+$ T cells may promote IgE production.

Key words: CD40 ligand, $CD8^+$ T cells, gene therapy, IgE production, interleukin-4.

Introduction

Adenosine deaminase (ADA) is a purine catabolic enzyme that catalyzes the conversion of adenosine and deoxyadenosine to inosine and deoxyinosine, respectively. The accumulation of intracellular adenosine and deoxyadenosine caused by a genetic defect in ADA leads to a disability of the human immune system, resulting in severe combined immunodeficiency characterized by defective T and B cell function.[1,2] On the basis of the development of gene therapy procedures for the treatment of genetic disorders, 11 patients with ADA deficiency have been enrolled in gene therapy protocols that have used different strategies, retroviral vector constructs and target cell populations.[3–7] The results obtained from these clinical trials have shown that gene transfer and expression successfully restore cellular and humoral

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immune responses. The patients enrolled in the gene therapy trials included a Japanese boy who received infusions of gene-corrected autologous T cells, the majority of which were positive for CD8. Quite unexpectedly, we found that he had an elevated serum IgE level and eosinophilia during the course.[8] We also showed that his circulating $CD8^+$ T cells expressed mRNA for some T helper 2 (Th2)-type cytokines following activation. Although such *in vivo* and *in vitro* observations have not been described in the other 10 patients, the data obtained in our patient raised the possibility that immunologic reconstitution with genetically modified $CD8^+$ T cells may contribute to increased IgE production and eosinophilia.

Although $CD8^+$ T cells play a role in protective immunity against viral infections and intracellular pathogens, recent studies have provided evidence that $CD8^+$ T cells support B cell antibody production, like $CD4^+$ T cells.[9–12] The $CD8^+$ cytotoxic T cells can be divided into two distinct cytokine-secreting subsets, Tc1 and Tc2, which are similar to the $CD4^+$ Th1 and Th2 cells.[13] The Tc1 cells produce Th1-type cytokines, such as interferon (IFN)-γ and lymphotoxin, while Tc2 cells, with reduced cytolytic activity, secrete Th2-type cytokines, including interleukin (IL)-4, IL-5 and IL-13. The Tc2 cells also provide a costimulatory signal for B cell isotype switching by expressing CD40 ligand (CD40L, CD154).[11,14] The $CD8^+$ T cells with a Tc2 phenotype have been described in patients with lepromatous leprosy, leishmaniasis, AIDS or atopy.[9,11,12,15,16] In addition to these patients, our ADA-deficient patient may have had Tc2-like $CD8^+$ T cells during the gene therapy clinical trial, as recently reported.[8] In the present study we analyzed the phenotypic and functional profiles of the patient's circulating lymphocytes in more detail.

METHODS

Gene therapy for an ADA-deficient patient

The present study was performed in a boy with ADA deficiency who received periodic infusion of culture-expanded autologous T cells that had been transduced with the human ADA cDNA containing the retroviral vector.[7] At the age of 4 years, the boy was enrolled in a clinical gene therapy trial and received a total of 11 infusions over a 20 month period. Before gene therapy, the patient had very low levels of $CD3^+$ cells (400 /μL), $CD4^+$ cells (205 /μL), $CD8^+$ cells (191 /μL) and $CD19^+$ cells (57 /μL) in his peripheral blood. Following commencement of the trial using genetically modified T cells, the patient's circulating $CD8^+$ cells increased gradually and accounted for approximately 90% of all $CD3^+$ cells after several infusions. This response has been sustained by the 11th infusion (CD3 1822 /μL; CD4 240 /μL; CD8 1538 /μL; CD19 154 /μL). Despite the marked inversion of the CD4/CD8 ratio, the patient had an elevated serum IgE test level, positive IgE radioallergosorbent (RAST) to house dust mites and eosinophilia. Details of the data have been described elsewhere.[8] In the present study, we conducted phenotypic and functional analyses of the patient's circulating lymphocytes obtained before each of the 8th to 11th infusions. Informed consent was obtained from his parents.

Cell preparation

Heparinized venous blood was taken from the patient and healthy adults with no history of allergy and also atopic adults with bronchial asthma or perennial allergic rhinitis. Peripheral blood mononuclear cells (PBMC) were separated by Ficoll–sodium metrizoate sedimentation (Organon Teknika Corp., Durham, NC, USA). Depletion of $CD2^+$, $CD4^+$, $CD8^+$, $CD14^+$, $CD19^+$ and/or $CD56^+$ cells from PBMC or the transduced cells was performed using magnetic beads coated with appropriate monoclonal antibodies (mAbs) or streptavidin (Dynal, Oslo, Norway), as described previously.[17] Purity of $CD20^+$ B cells obtained by treating PBMC was always > 98% as determined by flow cytometry. The $CD8^+$ T cell subset isolated from transduced cells contained no detectable $CD4^+$, $CD20^+$ or $CD56^+$ cells. The adult donors used for collection of peripheral blood gave informed consent to participate in the study.

Generation of $CD8^+$ T cell lines

The patient's PBMC were suspended at a concentration of 1×10^6 cells/mL in RPMI 1640 medium (Gibco BRL, Grand Island, NY, USA) with 10% fetal calf serum (FCS), 2 mmol/L L-glutamine, 100 U/mL penicillin and 100 μg/mL streptomycin. Cells were then stimulated with 1 μg/mL phytohemagglutinin (PHA)-P (Serva, Heidelberg, Germany) and 20 U/mL IL-2 (Genzyme Corp., Cambridge, MA, USA) in 24-well flat-bottomed plates (Becton Dickinson Labware, Lincolon Park, NJ, USA), as described in detail elsewhere.[9] In order to generate T cell lines, viable T blasts were cultured in bulk with the addition of IL-2. After analysis of the surface expression of

T cell markers, several $CD8^+$ T cell lines were grown and two cell lines containing the transgene were finally selected. The transgene-carrying cells were evaluated exactly as described previously.[7] Phenotypic analysis showed that both cell lines were positive for CD3, CD8 and T cell receptor (TCR)-α,β (> 99%), but negative for CD4, CD56 and TCR-γ,δ (< 1%). Lined $CD8^+$ T cells were rested in culture medium for at least 48 h after the last addition of IL-2.

Cytokine assays

Immunoreactive IFN-γ, IL-4 and IL-13 were quantitatively assayed with commercially available ELISA kits (BioSource International, Camarillo, CA, USA) according to the manufacturer's instructions. The sensitivity of each assay was 7.8 pg/mL.

Flow cytometric analysis

For one-color immunofluorescence staining, cells were labeled with appropriate concentrations of fluorescein isothiocyanate (FITC)/phycoerythrin (PE)-conjugated mAbs or incubated with biotinylated mAb and PE-conjugated streptavidin. For two-color immunofluorescence staining, cells were labeled with two different mAbs conjugated with FITC or PE, or incubated with biotinylated mAb followed by PE-conjugated streptavidin and FITC-conjugated mAb. The mAbs used were anti-CD3, anti-CD4, anti-CD8, anti-CD20, anti-HLA-DR (all from Becton Dickinson, San Jose, CA, USA), anti-CD40 (Serotec, Oxford, UK), anti-CD80, anti-CD86 (two from PharMingen, San Diego, CA, USA), anti-Fas (anti-CD95, Medical & Biological Laboratories, Nagoya, Japan), anti-CD40L (anti-CD154; Ancell, Bayport, MN, USA), anti-FasL (anti-CD95L; Transduction Laboratories, Lexington, KY, USA) and anti-CD23 that was the same as described previously.[17] Isotype-matched control mAbs were used for negative staining. Stained cells were analyzed by flow cytometry on a FACScan (Becton Dickinson) using a gate for lymphocytes.

Spontaneous and IL-4-induced production of IgE

Measurement of IgE synthesis was conducted as described previously.[17] The patient's PBMC were suspended at a concentration of 1×10^6 cells/mL in Iscove's modified Dulbecco's medium (Gibco BRL) supplemented with FCS, L-glutamine and antibiotics (culture medium). Aliquots of the cell suspension in a total volume of 0.2 mL were cultured in 96-well round-bottomed plates (Coastar Corp., Cambridge, MA, USA) with or without 200 U/mL IL-4 (Genzyme Corp.) for 14 days. To estimate preformed IgE, both cycloheximide and puromycin were added to some cultures. The concentration of IgE in culture supernatants was measured by an isotype-specific radioimmunoassay. Net IgE synthesis was calculated by subtracting the value of preformed IgE.

Coculture experiments

Transduced or lined $CD8^+$ T cells at a density of 1×10^6 cells/mL were stimulated for 24 h with or without 100 ng/mL anti-CD3ε mAb (PharMingen), followed by fixation with 0.5% paraformaldehyde. Fixed cells (1×10^5 cells) were incubated in the presence of 200 U/mL IL-4 with purified normal human B cells (1×10^5 cells) in a total volume of 0.2 mL in culture medium. Aliquots of the cell suspension were dispensed into 96-well round-bottomed plates and cultured for either 3 (B cell proliferation) or 14 days (IgE synthesis). For the B cell proliferation assay, cultures were pulsed for 12 h with [^{3}H]-thymidine (18.5 kBq/mL; Amersham Japan, Tokyo, Japan). In some experiments, wells had a chimeric molecule (20 μg/mL) generated by fusing the extracellular domain of human CD40 to the heavy chain of human IgM (soluble form of CD40; kindly provided by Dr P Lane, Basel Institute for Immunology, Basel, Switzerland).[18]

Reverse transcription–polymerase chain reaction analysis

Extraction of total cellular RNA, cDNA synthesis by reverse transcription (RT) and polymerase chain reaction (PCR) were performed as described previously.[19] The 5′ sense primers and 3′ antisense primers used for amplification of mRNA for IL-4, IL-13, CD40L, FasL, germline Cε, mature Cε and glyceraldehyde 3-phosphate dehydrogenase (G3PDH) were the same as described previously.[17] Primer pairs specific for IFN-γ were obtained from Clontech Laboratories (Palo Alto, CA, USA). The reactions were all within the linear range. The PCR products were separated on a 2% agarose gel and stained with ethidium bromide.

RESULTS

Phenotypic and functional analyses of PBMC from an ADA-deficient patient treated with gene therapy

An ADA-deficient patient received a total of 11 infusions of transduced T cells. Before each of the last four infusions,

PBMC were isolated from the patient, stained with mAbs and analyzed by flow cytometry. The results of cell surface marker analysis performed before the 8th gene therapy are shown in Fig. 1a. The PBMC contained CD3+ T cells with a CD4/CD8 ratio of approximately 0.1. This marked inversion was sustained by the 11th gene therapy (data not shown). Both $CD4^+$ and $CD8^+$ T cell subsets were negative for CD40L and FasL, while $CD20^+$ B cells that remained in the normal range constitutively expressed CD40 and human lymphocyte antigen (HLA)-DR, but no CD80, CD86 and Fas. Interestingly, CD23 expression was detected on the majority of $CD20^+$ B cells. When stimulated for 48 h with 100 U/mL IL-4, purified B cells responded by upregulation of CD23, CD40, HLA-DR and CD86 (Fig. 1b). Although culture alone induced CD86 expression, this may be caused by FCS stimulation.

Both germline and mature Cε transcripts could be detected by RT-PCR analysis of fresh PBMC obtained before the 8th gene therapy (Fig. 2a). These two transcripts were detected in fresh PBMC of an atopic subject, but not in those of a healthy donor. *In vivo* expression of germline and mature Cε mRNA in the patient's PBMC was sustained throughout the study (Fig. 2b). Furthermore, the PBMC obtained before each of the 8th to 11th gene therapy sessions spontaneously secreted detectable amounts of IgE and responded to IL-4 with increased IgE production (Fig. 3). The concentration of IgE produced by the patient's PBMC stimulated with IL-4, which ranged from 6.6 to 12.8 ng/mL, approximated that produced by IL-4-stimulated PBMC from healthy donors (mean (± SEM) 9.7 ± 2.2 ng/mL; $n = 15$). Although these values were somewhat low compared with those obtained in cultures of PBMC from atopic donors (17.0 ± 3.9 ng/mL, $n = 22$), IgE levels spontaneously secreted by the patient's PBMC, which ranged from 0.3 to 1.9 ng/mL, were almost equal to those secreted by unstimulated PBMC from 22 atopic donors (2.3 ± 0.9 ng/mL).

Collectively, these results demonstrate that the ADA-deficient B cells of the patient were functionally mature with respect to CD23 expression, germline and mature

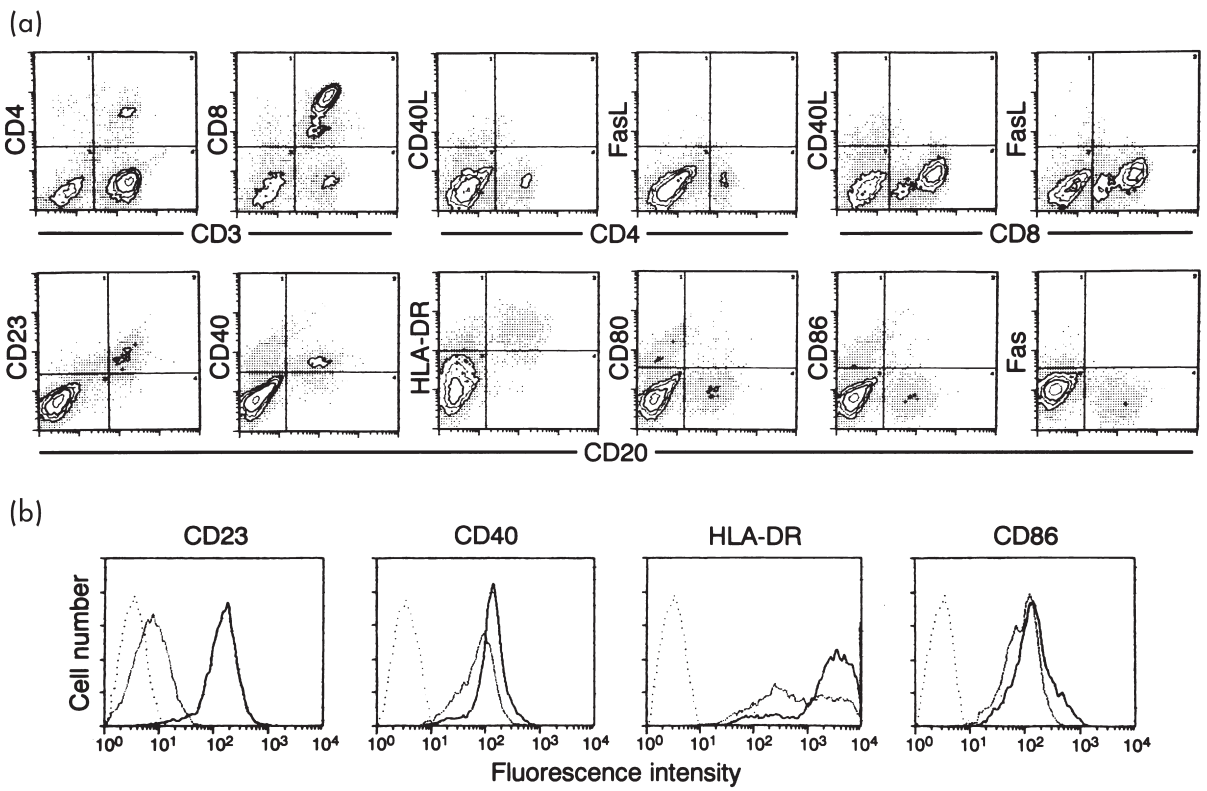

Fig. 1 Analysis of the patient's circulating lymphocytes obtained before the 8th gene therapy session. (a) Two-color staining of fresh peripheral blood mononuclear cells (PBMC) was done. (b) Purified B cells were cultured for 48 h with medium alone (·····) or interleukin (IL)-4 (——) and were singly stained. In each experiment, background staining was obtained by incubating cells with isotype-matched control monoclonal antibodies (mAbs; ····· in (b)). Cells were analyzed by flow cytometry using mAbs to the indicated cell surface markers. Similar results were obtained in the patient's PBMC and B cells obtained before the 9th to 11th gene therapy sessions (data not shown).

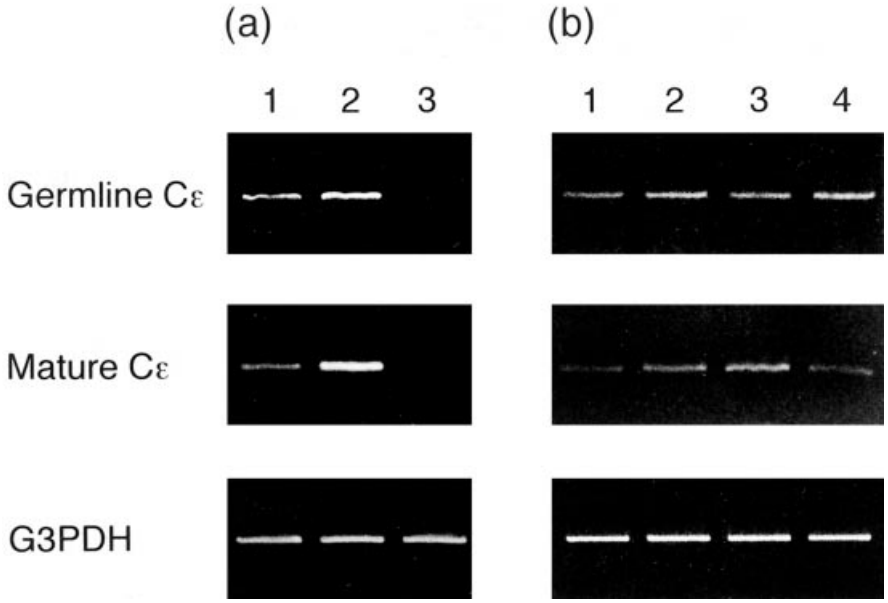

Fig. 2 Analysis of *in vivo* expression of germline and mature Cε transcripts in fresh peripheral blood mononuclear cells (PBMC). (a) Comparison of germline and mature Cε mRNA expression in the patient's PBMC obtained before the 8th gene therapy session (lane 1) and PBMC from an atopic subject (lane 2) and a healthy donor (lane 3). (b) Expression of germline and mature Cε mRNA in the patient's PBMC obtained before the 8th (lane 1), 9th (lane 2), 10th (lane 3), and 11th (lane 4) gene therapy sessions. In each experiment, total cellular RNA was prepared and the expression of mRNA for germline Cε, mature Cε and glyceraldehyde 3-phosphate dehydrogenase (G3PDH) was analyzed by reverse transcription–polymerase chain reaction.

Ce transcription and IgE synthesis, despite the marked inversion of the CD4/CD8 ratio after gene transduction procedures.

CD8+ T cells from gene-transduced T cells

CD8+ T cells were purified from transduced T cells used for the 8th infusion, but no selection procedure to enrich for gene-transduced CD8+ T cells was performed. Cells were stimulated with or without anti-CD3ε mAb and were analyzed for the capacity to express mRNA for IFN-γ, IL-4, IL-13, CD40L and FasL. As summarized in Fig. 4a, IFN-γ mRNA, which was detected even in unstimulated cells, increased at 3 h after stimulation and accumulated at relatively high levels to 24 h. Interleukin-4 mRNA expression was transient, beginning as early as within 1 h after stimulation and declining thereafter. In contrast, dual expression of IL-13 mRNA was induced after stimulation: the first peak at 1 h and the second at 12 h. Induction of CD40L mRNA occurred within as little as 1 h of stimulation, reached a peak at 12 h and declined thereafter. The increase in FasL mRNA also occurred at 6 h after stimulation. These observations are in keeping with the data obtained at the protein level, where unstimulated cells secreted only IFN-γ, while stimulated cells produced IFN-γ in larger quantities together with detectable amounts of IL-4 and IL-13 (Fig. 4b) and expressed surface CD40L and FasL at almost the same level (Fig. 4c). Similar results

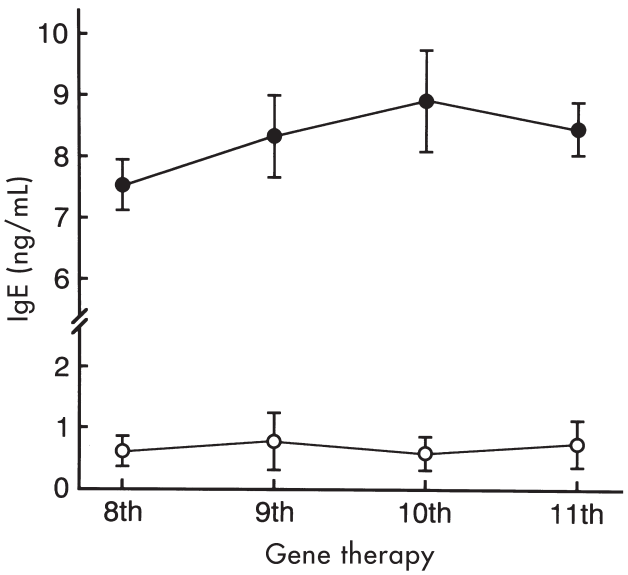

Fig. 3 Spontaneous and interleukin (IL)-4-induced production of IgE by the patient's peripheral blood mononuclear cells (PBMC). Cells obtained before each of the 8th to 11th gene therapy sessions were cultured for 14 days in the absence (○) or presence (●) of IL-4. The concentration of IgE in culture supernatants was measured by an isotype-specific radioimmunoassay. Net IgE synthesis was determined by subtracting the value of preformed IgE. Results are the mean ± SEM of triplicate cultures.

were obtained in CD8+ T cells from transduced cells used for the 9th, 10th and 11th infusions (data not shown).

These results suggest that the properties of CD8+ T cells from the transduced cells may be similar to those of Th0-type CD4+ T cells.

Lined CD8+ T cells from peripheral T cells carrying the transgene

Two lined CD8+ T cells containing the transgene were generated from PBMC obtained before the 10th gene therapy session, but no selection procedure to enrich for transgene-expressing CD8+ T cell lines was performed. Such lined CD8+ T cells were cultured in the absence or presence of anti-CD3ε mAb for 24 h and analyzed for the capacity to produce IFN-γ, IL-4 and IL-13 and to express CD40L and FasL. A representative result is shown in Fig. 5. After being stimulated, cytokine production increased in the order of IFN-γ < IL-4 < IL-13 (Fig. 5a). Surface expression of CD40L was also detected on stimulated cells, whereas induction of FasL expression was very weak (Fig. 5b). These results imply that, to some extent, lined CD8+ T cells containing the transgene exhibit a Tc2-like phenotype.

B Cell proliferation and IgE synthesis induced by CD8+ T cells from transduced cells and cell lines

Before the 10th gene therapy session, both transduced and lined CD8+ T cells were prepared as described. These two kinds of CD8+ T cells were stimulated with or without anti-CD3ε mAb, fixed in paraformaldehyde and added to normal human B cells, followed by culture in the presence of IL-4. The results are summarized in Fig. 6. Although none of the unstimulated CD8+ T cells had any ability to help B cells, stimulated lined cells provided B cell proliferation and help for IgE synthesis more effectively than did stimulated transduced cells. Addition of a soluble form of CD40 to the cultures led to abrogation of B cell proliferation and IgE production in each case, indicating the involvement of functional CD40L. Unlike in lined CD8+ T cells, the optimal concentration of transduced CD8+ T cells for inducing IgE synthesis was 50%, with higher concentrations of the cells being inhibitory (data not shown). These results demonstrate that CD40L-dependent B cell help provided by lined CD8+ T cells was

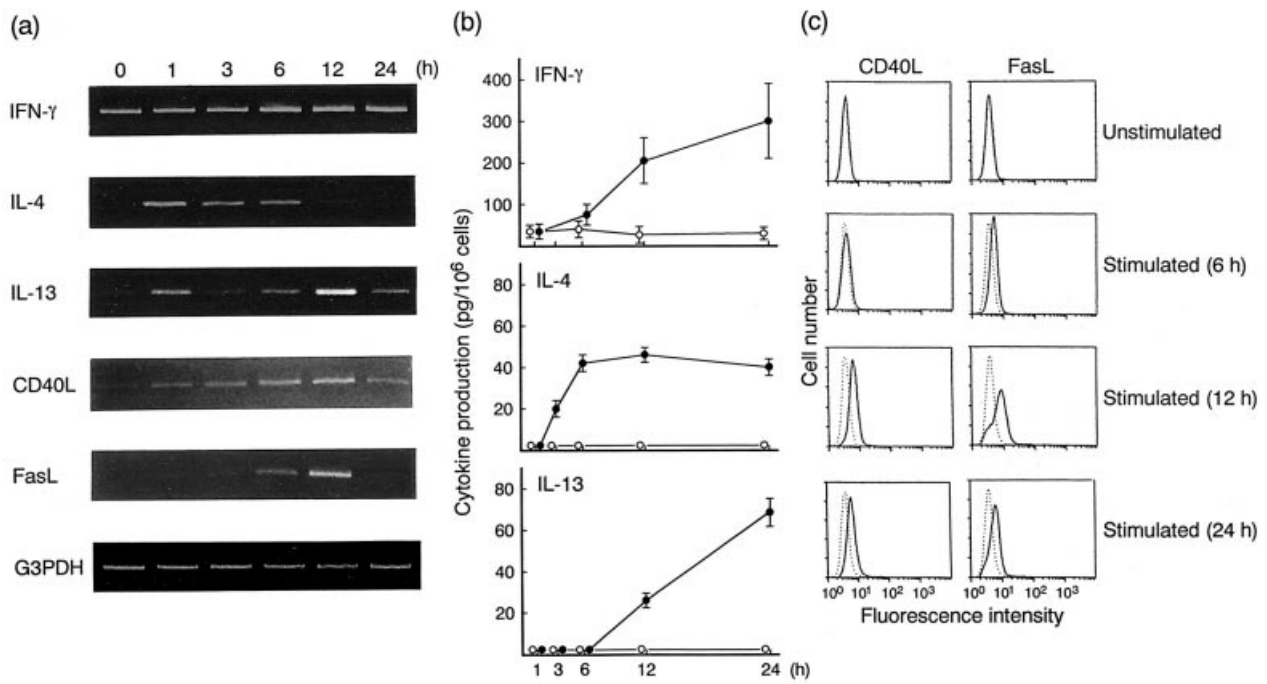

Fig. 4 Analysis of CD3-mediated expression of mRNA and protein for interferon (IFN)-γ, interleukin (IL)-4, IL-13, CD40L and FasL in CD8+ T cells transduced with the adenosine deaminase (ADA) cDNA. The CD8+ T cells from transduced cells at the 10th infusion were stimulated with or without anti-CD3ε monoclonal antibody (mAb) and cells and supernatants were harvested at the indicated time point. (a) After extraction of total cellular RNA, mRNA for the indicated molecules was amplified by reverse transcription–polymerase chain reaction (RT-PCR). The PCR products were subjected to electrophoresis and were visualized by ethidium bromide. (b) Supernatants were measured for cytokine concentrations by ELISA kits. Each point represents the mean ± SEM of triplicate cultures. (c) Cells were analyzed by flow cytometry using mAbs to the indicated cell surface markers.

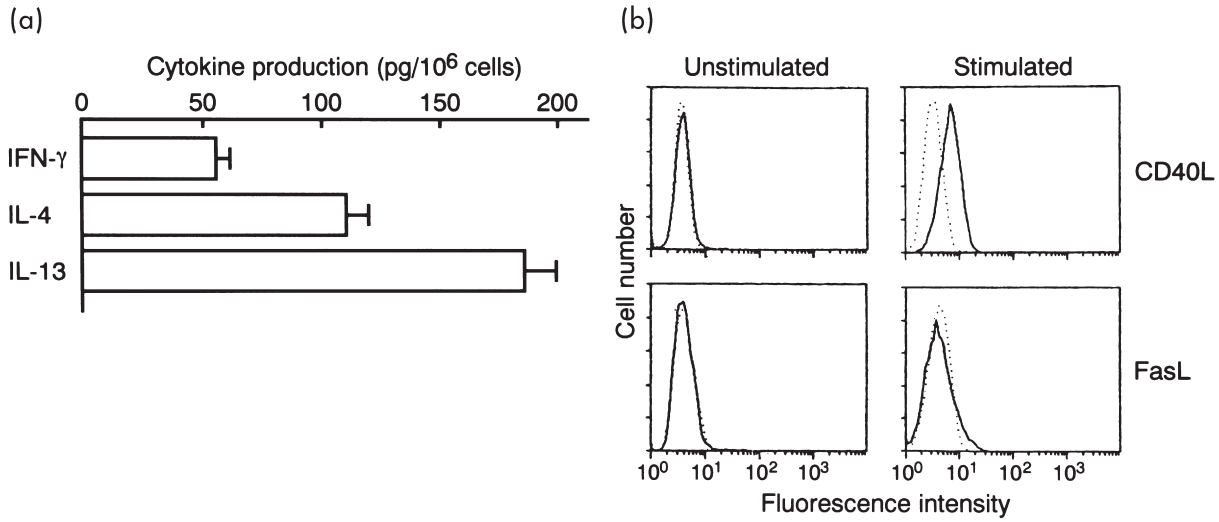

Fig. 5 Production of interferon (IFN)-γ, interleukin (IL)-4 and IL-13 and expression of CD40L and FasL by lined $CD8^+$ T cells from the patient's peripheral blood mononuclear cells obtained before the 10th gene therapy session. Lined $CD8^+$ T cells were stimulated for 24 h with or without anti-CD3ε monoclonal antibody (mAb) and supernatants and cells were harvested. (a) Supernatants were measured for cytokine concentrations by ELISA kits. Results are the mean ± SEM of triplicate cultures. Unstimulated cells produced no detectable cytokines (data not shown). (b) Cells were analyzed by flow cytometry using mAbs to the indicated cell surface markers.

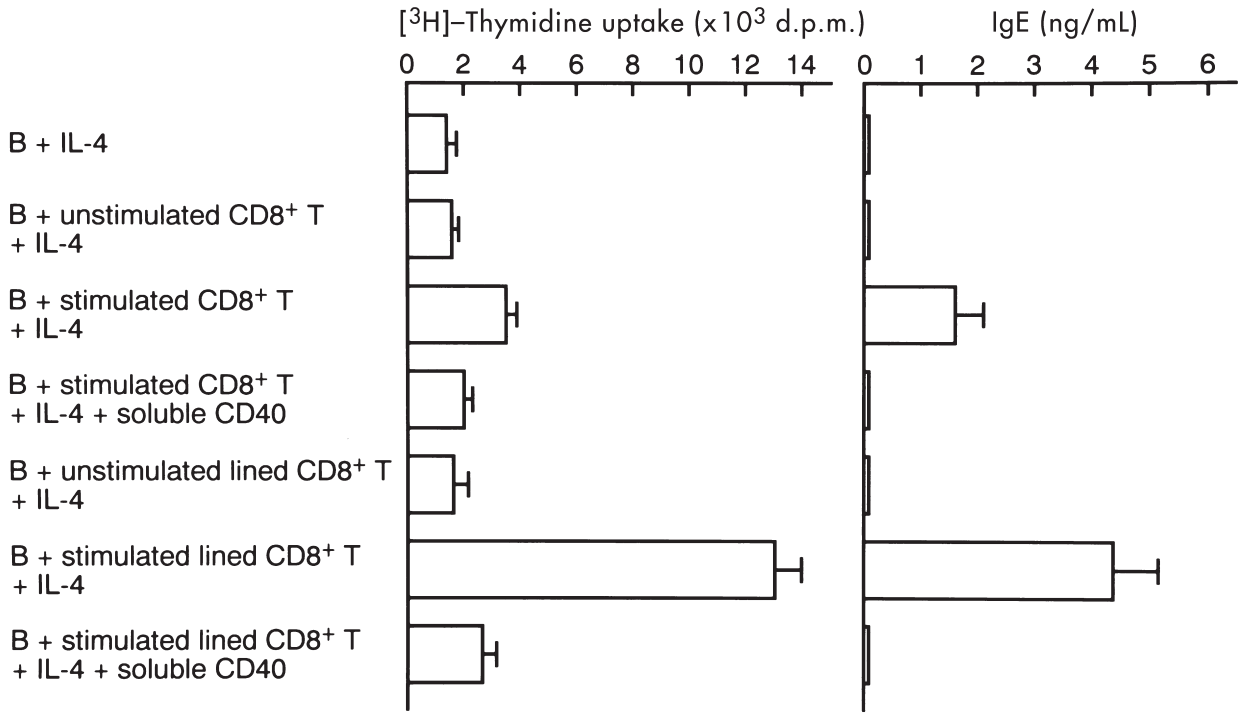

Fig. 6 B Cell proliferation and IgE production induced by $CD8^+$ T cells transduced with the adenosine deaminase (ADA) cDNA and by lined $CD8^+$ T cells from peripheral T cells carrying the transgene. These two $CD8^+$ T cells were prepared before the 10th gene therapy session, stimulated for 24 h with anti-CD3ε monoclonal antibody and fixed with paraformaldehyde. Normal human B cells were incubated in the presence of interleukin (IL)-4 with transduced or lined $CD8^+$ T cells for either 3 (B cell proliferation) or 14 days (IgE production). After culture, [^{3}H]-thymidine uptake and IgE concentrations were measured. Results are the mean ± SEM of triplicate cultures.

potent compared with that provided by transduced CD8+ T cells.

Discussion

In the present study, we investigated the mechanisms responsible for the elevation of serum IgE levels observed in an ADA-deficient patient treated with T cell-directed gene therapy. On the basis of the observation that this clinical trial led to the marked inversion of the CD4/CD8 ratio due to a favorable balance for CD8+ T cell gene transduction,[7] we analyzed the functional profile of the patient's CD8+ T cells obtained during the course of the trial. The experiments presented in this study demonstrate that immunologic reconstitution with genetically modified CD8+ T cells may promote the generation of Tc2-like cells that contribute to IgE production by B cells.

When stimulated with anti-CD3ε mAb, CD8+ T cells from transduced T cells produced IFN-γ, together with smaller amounts of IL-4 and IL-13, and expressed comparable levels of CD40L and FasL, while lined CD8+ T cells from circulating T cells carrying the transgene produced IL-4 and IL-13, together with smaller amounts of IFN-γ, and preferably expressed CD40L rather than FasL. Our data also show that the level of CD40L induction by the stimulated lined CD8+ T cells correlates with their level of IL-4 and IL-13 production and with a reduction of FasL expression. Because CD8+ T cell clones without the capacity to secrete IL-4 have been reported to fail to express CD40L,[10] it is conceivable and probable that transduced and lined CD8+ T cells have Th0- and Tc2-like phenotypes, respectively. Although the possibility that transduced CD8+ T cells contained a mixed population of Tc1 and Tc2 cells cannot be ruled out, supernatants from transduced CD8+ T cells stimulated with anti-CD3ε mAb, as well as those from the stimulated lined CD8+ T cells, were able to induce germline Ce transcription in normal human B cells (data not shown). These observations indicate that following immunologic stimualtion, both transduced and lined CD8+ T cells produce biologically active IL-4 and/or IL-13.

Furthermore, after stimulation and fixation, lined CD8+ T cells, in conjunction with the presence of IL-4, provided CD40L-dependent B cell help more effectively than did transduced CD8+ T cells. The dose–response curve for CD8+ T cell-induced IgE synthesis showed an optimum at a concentration of 50% transduced CD8+ T cells, with higher concentrations of cells being inhibitory, unlike lined CD8+ T cells. This may be due to differences in FasL expression between transduced and lined CD8+ T cells. Actually, it has been shown that FasL expression by subsets of CD4+ and CD8+ T cells is relatively restricted to Th0, Th1 and Tc1.[20–23] Although we did not examine directly the IgE-inducing activity of the viable stimulated CD8+ T cells, the observation that both transduced and lined CD8+ T cells produced biologically active IL-4 and IL-13 and expressed functional CD40L in response to anti-CD3ε mAb, together with the finding that purified CD8+ T cells from the patient's PBMC secreted Th2-type cytokines following PHA stimulation,[8] supports the notion of a stimulatory effect of Tc2-like CD8+ T cells on IgE production by B cells. This may be one of the possible mechanisms contributing to the elevation of serum IgE levels in our patient. Because polyethylene glycol (PEG)-ADA was maintained at the same dosage as before gene therapy,[7] it is also possible that enhancement of *in vivo* IgE production may result from a combination of gene therapy and PEG-ADA. However, it should be emphasized that PEG-ADA treatment alone was ineffective in increasing serum IgE levels.[8] These findings strongly suggest the possibility that genetically modified CD8+ T cells play an important role in acting as a trigger for the induction of IgE synthesis.

Differential expression of transcription factors, adhesion molecules and chemokine receptors has been shown in polarized Th1 and Th2 cells.[24–29] With regard to Th2-specific transcription factors, c-MAF and GATA-3 are thought important in transactivating the IL-4 promoter. In contrast to GATA-3, which is also expressed in Th0 cells, c-MAF is selectively expressed in polarized Th2 cells, particularly in murine CD4+ T cells. Although the human c-maf sequence has not yet been available, mouse, rat and chicken have *c-maf* with a high degree of sequence homology.[24] To examine c-maf expression in IL-4-producing human CD8+ T cells, we conducted RT-PCR analysis using primer pairs specific for conserved areas with almost complete sequence homology with mouse and chicken c-maf. Our preliminary results showed that both transduced and lined CD8+ T cells, as well as the murine Th2 clone D10G4.1, constitutively expressed detectable c-MAF transcripts (K Ikizawa *et al*., unpubl. obs., 1999). However, recent studies in human T cells have demonstrated that the c-MAF binding site does not appear essential for IL-4 promoter activity[30] and that Th1 and Th2 clones have comparable levels of c-maf expression.[29] These findings suggest that c-maf expression may not always reflect the generation of IL-4-producing human T cells, including CD4+ and CD8+ T cell subsets. The

pattern of GATA-3 and chemokine receptor expression in the two CD8+ T cells used in the present study is currently being analyzed.

In our patient, transduced CD8+ T cells differentiated into Tc2-like cells after infusion. Indeed, lined CD8+ T cells from peripheral T cells carrying the transgene produced IL-4 and IL-13 and expressed CD40L following stimulation with anti-CD3ε mAb. This may explain why immunologic reconstitution with genetically modified CD8+ T cells led to enhanced IgE production. Furthermore, because the frequency of ADA cDNA-integrated cells in peripheral T cells and immunologic improvement showed a good correlation,[31] it is most likely that transduced, rather than untransduced, CD8+ T cells play a major role in helping B cells to produce IgE. In contrast, there remains the possibility that the very small CD4+ T cell population in the transduced cells also underwent *in vivo* activation to provide help for B cells in IgE production. Further investigations are necessary to analyze the contribution of transduced CD4+ T cells to the regulation of IgE synthesis.

In summary, we show that preferential gene transduction into CD8+ T cells allows the development of Tc2-like cells that provide help for B cells in IgE synthesis by producing IL-4 and IL-13, together with smaller amounts of IFN-γ, and by preferably expressing CD40L rather than FasL. These findings support the notion of a high frequency of CD8+ T cells with a Tc2 phenotype in some diseases showing elevated serum IgE levels.

Acknowledgments

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