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Novel Insight into the Agonistic Mechanism of Alefacept In Vivo: Differentially Expressed Genes May Serve as Biomarkers of Response in Psoriasis Patients

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Alefacept is an LFA3-Ig fusion protein that binds to CD2 and is thought to inhibit T cell activation by antagonism of CD2 signaling or by lysis of CD2+ cells. Alefacept is potential future therapeutic for organ transplant recipients or graft-vs-host disease and is an approved therapeutic for psoriasis vulgaris, which is a T cell-mediated inflammatory disease. However, alefacept improves psoriasis in only ~50% of patients treated for 12 wk. We studied the immunologic effects of alefacept in a group of psoriasis patients during treatment. We found that T cells, especially CD8+ T cells, were rapidly decreased in the peripheral circulation. Decreases in circulating T cells were not associated with induced apoptosis. Unexpectedly, in addition to suppression of inflammatory genes, we found a marked induction of mRNAs for STAT1, IL-8, and monokine induced by IFN-γ during the first day of treatment in PBMC. We confirmed the agonistic effects of alefacept in PBMC in vitro, which were similar to CD3/CD28 ligation on T cells. These data establish that alefacept activates gene expression programs in leukocytes and suggest that its therapeutic action may be as a mixed agonist/antagonist. Furthermore, responding patients to alefacept treatment show unique patterns of gene modulation. Whereas alefacept down-regulated TCRs CD3D and CD2 in responders, nonresponders reveal a higher expression of T cell activation genes such as CD69 in pretreatment PBMC. These finding suggest a potential basis for categorizing responders vs nonresponders at an early time point in treatment or before treatment of a broad range of proinflammatory diseases. This study 1) establishes alefacept as a novel CD2 agonist molecule for induction of leukocyte activation genes (prior work proposed its mechanism as a CD2 antagonist) and 2) that differential activation of genes may categorize clinical responders to this agent, critical for cost-effective use of this drug. The Journal of Immunology, 2007, 178: 7442–7449.

LYmphocyte functional Ag-1 and CD2 have emerged as important therapeutic targets for the treatment of psoriasis vulgaris, a common immune-mediated inflammatory disease of the skin that affects ~25 million people in North America and Europe. Currently approved therapeutics to these targets are efalizumab, a humanized mAb that binds to the CD11a subunit of LFA-1 (1), and alefacept, a LFA3-Ig fusion protein that binds to CD2 (LFA-2) (2). CD2 is expressed on the surface of all T cells, but at relatively higher levels on memory and activated T cells (3). CD2 binds to its counterreceptor CD58/LFA-3, which is expressed mainly on APCs. This interaction is thought to increase T cell activation to specific Ags by lowering the required threshold concentration of Ag presented to T cells by APCs (4). In vitro ligation of CD2 with certain combinations of Abs (such as mAbs against T112 plus T113) can trigger T cell proliferation, independent of TCR-mediated signals, so CD2 is considered a T cell surface molecule with the potential for delivering agonist or activation signals to the T cell (5). However, the biology of CD58:CD2 interactions has been difficult to study, as expression of CD58 is restricted to humans and primates. Mouse T cells do express CD2, but the binding interaction is with CD48, a molecule that has much lower affinity for CD2 compared with CD58. Even so, Abs to CD2 in murine models of transplantation or inflammation have been used successfully to attenuate T cell-mediated effector responses (6, 7).

Alefacept is a human recombinant fusion protein comprised of the LFA3 (CD58) extracellular domain fused to the hinge CH2 and CH3 (c-terminal constant) regions of a human IgG1 (8). Like certain CD2 Abs (9), alefacept was shown to inhibit T cell proliferative responses to various Ags or mitogens and was designated a T cell inhibitory protein (LFA3TIP). The Ig domain of alefacept interacts with cellular FcR, which is critical for in vitro T cell inhibition. FcR-mediated bridging of NK cells with T cells has lead to elimination of bound T cells by induced cell death in vitro (10).

Based on in vitro models, two therapeutic mechanisms have been suggested for disease improvement in psoriasis mediated by alefacept: 1) deletion of memory T cells mediated by FcR bridging with activated NK cells (11) or 2) inhibition of CD2-mediated T cell costimulation via interference with CD58 binding on APCs (8). Although these therapeutic mechanisms are both tenable, there is little evidence to support them in vivo. If NK cells induced death of memory T cells, there should be a good correlation between reduction in memory CD4+ T cells and clinical response. In fact, this is a weak correlation (12). Considering the second hypothesis,
were incubated for 15 min with AnnexinFITC, propidium iodide (PI) (BD Pharmingen) according to manufacturer’s protocol. In brief, PBMC with the following Abs:

PBMC were isolated and stored at −80°C until required as previously described (14). Briefly, mRNA was isolated to measure the gene expression with microarray analysis.

In vivo and in vitro assays

Microarray analysis. U95Av2-set GeneChip probe microarrays (Affymetrix) were used containing probe sets representing ~12,000 genes. Fragmentation and array hybridization were conducted according to manufacturer’s instructions (Affymetrix; RiboAmpOA, RNA Amplification kit; Arcturus). Scanning and quality control, GeneChip expression value analysis, hierarchical clustering, and heat maps were performed as previously described (15). Briefly, for individual transcripts, fold change in expression was the ratio of the mean expression level in each condition in vivo pretreated vs 6 h posttreatment: n = 17 for all patients (sufficient good quality RNA was available for 17 of total 22 patients), n = 9 for responders, and n = 8 for nonresponders. Similarly, in vitro-treated PBMC after anti-CD3/CD28 activation or alefacept treatment were compared with non-stimulated controls. A heat map of the computed tree represents unsupervised hierarchical clustering and is presented as red and green lines. Each line presents the relatively up-regulated (red) or down-regulated (green) expression value of the genes in fold changes. Complete lists of genes with p values and description of relevant function of genes are provided in supplemental Table I. GeneOntology annotations of differentially expressed genes were collected from LocusLink (www.ncbi.nlm.nih.gov/LocusLink).

Validation of expression changes in mRNA with real-time RT-PCR analysis.

The expression of the following genes was tested in PBMC: IL-8, STAT1, monokine induced by IFN-γ (MIG), human acidic ribosomal protein (HARP), granulocyte M (GZMB), granzyme B, IFN-gamma (IFN-γ), forkhead box P3 (Foxp3), RANTES (CCL5), CD33, CD2, CD8A, TRAIL, STAT4, CD3D, CD7, IL-7R, forkhead box P3 (Foxp3), and MyD88. IFN-γ and iNOS were tested in skin biopsies. The kR gene, a housekeeping gene, was used to normalize each gene. The primers and probes for these genes for the TaqMan RT-PCR assays were generated with the Primer Express algorithm, version 1.0, using published genetic sequences (National Center for Biotechnology Information-PubMed) for each gene. The primer sequences have been published for IL-8, STAT1, MIG, HARP (14), and GZMB (15). The primers sequence was as follows for: CD122; forward CAACACCCCGCTGTGATCC; reverse CTTCCTCCTGGCAAATGGG; CD122 probe: 6FAM-TGCCATGAGCTAGAAGCATCACAGGTTGA-TAMARA (GenBank accession number NM_001781); 6FAM-TGCCATGAGCTAGAAGCATCACAGGTTGA-TAMARA (GenBank accession number NM_001781); RANTES (CCL5); forward CGTCCATCGCTCCTCCCA, CCL5 reverse CTGGCAATGACAGCAGGCTGACCAACACCGCCCTTGA-TAMARA (GenBank accession number NM_001781); RANTES (CCL5); forward CGTCCATCGCTCCTCCCA, CCL5 reverse CTGGCAATGACAGCAGGCTGACCAACACCGCCCTTGA-TAMARA (GenBank accession number NM_001781); MyD88, CD69, and iNOS were tested in skin biopsies. The kR gene, a housekeeping gene, was used to normalize each gene.

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Statistical analysis

Statistical comparisons of expression level for flow cytometry and real-time RT-PCR data were pairwise between pretreatment data and during treatment. A two-tailed Student’s t test was used; significance was designated as \( p < 0.05 \).

Statistical comparisons of microarray data involved initially performing a robust multiple chip analysis to normalize the expression, followed by Student’s t test to assess significantly different genes in pretreatment and 6 h posttreatment on all genes present. This generated a biologically interesting gene list for further analysis. Due to multiple testing in the analysis of microarray data, we chose to declare those genes with a false discovery rate of at most 5% as being significantly affected. Significant levels were adjusted for multiplicity by the Benjamini-Hochberg method.

Results

Alefacept binding parallels CD2 expression on naive and memory T cells

We analyzed the CD2-binding properties of alefacept on different populations of T cells. A variety of different leukocyte markers (Fig. 1A) were used to quantify naive (CD45RA+) T cell populations and memory (CD45RO+) T cell populations. The lowest expression of CD2 and lowest alefacept binding were in naive CD4+ T cells, while the highest CD2 expression and highest alefacept binding occur in memory CD8+ T cells. Overall, alefacept binding was parallel to CD2 expression on naive vs memory T cells.

Alefacept preferentially and persistently decreases circulating CD88+ T cells

Following administration of alefacept, there is a rapid and dramatic reduction of peripheral T cells compared with B cells or NK cells (Fig. 1B). T cells (CD3+) were the key population of lymphocytes showing a more dramatic decrease in cell numbers as compared with NK cells. Only slight changes were seen in B cells. We further differentiated the effects of alefacept on CD4+ and CD8+ T cell populations by measuring the absolute counts of CD3+, CD4+, and CD8+ T cells (Fig. 1C). Alefacept decreased CD3+ T cells as early as 1 h posttreatment (34.5% reduction, \( p < 0.01 \)) and the levels stayed low after 13 wk of treatment (29.5% reduction, \( p < 0.01 \)). The dominant target for alefacept appears to be the CD88+ T cell population. Alefacept reduced the CD88+ T cell population at 1 h posttreatment by 49.64% (\( p < 0.01 \)). Reductions were consistent over 13 wk of treatment. The CD4+ population was affected at 1 h (31.26% reduction, \( p < 0.05 \)) but partially recovered after week 4. Table I describes the reduced numbers of CD88+ T cells and CD4+ T cells over the treatment time points. The percentage reduction is compared with pretreatment. The confidence intervals between the two populations of CD4+ and CD88+ T cells overlap minimally as also evident by the significant differences in the average changes (\( p < 0.02-0.0001 \)). At week 13, memory CD4+ T cells were decreased by a mean of 40% (\( p < 0.001 \)), whereas memory CD8+ T cells were decreased by a mean of 65% (\( p < 0.001 \)) (data not shown).

Alefacept treatment rapidly alters gene expressions in PBMC in psoriasis patients in vivo

To understand the effects of alefacept treatment on genes expressed in peripheral leukocytes, we analyzed gene expression of proinflammatory genes in PBMC collected from psoriasis patients during a clinical trial with alefacept therapy. To our surprise, we detected a large increase in gene expression of proinflammatory genes STAT1 and MIG within the first day of treatment, but peaking at 6 h posttreatment (Fig. 2). The effect on gene activation was rapid, as by week 13 of treatment, the gene expression normalized to baseline values (Fig. 2).

To detect how alefacept might be affecting gene expression in general at this early time point, we tested expression of 12,000 genes using Affymetrix human U95A+2 gene chips at baseline and after 6 h of treatment and Fig. 3A shows a heat map of genes with increased expression at 6 h; Fig. 3Ai shows genes with suppressed expression at 6 h. The complete description of the genes is available online in supplemental Table Ia. Genes with up-regulated expression compared with levels of the same genes expressed in pretreatment samples are indicated in red; genes that are reduced

FIGURE 1. Alefacept binds to CD2 and causes a reduction in CD3+, CD4+, and CD88+ T cells. A, CD2high cells correlate closely with LFA3TIP binding in various populations of circulating T cells. Histogram FACS plots of CD2 and LFA3TIP binding for a psoriasis patient at baseline; “high” populations indicated by bar. Histogram for CD2 expression (i) and LFA3TIP-PE binding (ii) for naive (CD45RA+), memory (CD45RO+) CD4+ T cells, naive (CD27CD45RA), and memory (CD27CD45RA) CD88+ T cells. Mean fluorescence intensity indicated for CD2 or LFA3TIP binding to each population on the histogram. Appropriate isotype histogram is shown (line) on the left of each row. B, Decrease in total circulating T cells during alefacept treatment of psoriasis (n = 22). CD3+ T cells, CD19+ B cells, and CD56/16– NK cells before (pre) and during the course of therapy. C, Alefacept preferentially modulates circulating CD88+ T cells during the course of therapy. CD3+ T cells, CD88+ T cells, and CD4+ T cells before and during the course of therapy are shown. Mean absolute counts are shown at each time point. SEM, \( p < 0.05 \).
was both an increase (Fig. 3) and a decrease (Fig. 3B), subsequent increase in transcription factors other treatments of psoriasis such as cyclosporine A (Fig. 4) described (14). This is a unique effect of alefacept compared with decreased expression of the proinflammatory genes, as previously stages of therapy. At week 13 posttreatment patients showed a inflammatory mediators and their primary cellular sources at early primarially by T cells, and there is clearly a different effect on these up-regulated within 6 h, including MIG. There are several genes of this “type 1” pathway that are leading to induction of downstream cytokines such as IL-8 and MIG. There are several genes of this “type 1” pathway that are regulated within 6 h, including IL-8, STAT1, and MIG (Fig. 3, Ai and Bi), which is opposite to what we would have predicted with this therapy (14). Gene expression data suggest that alefacept possibly could increase expression of inflammatory genes in leukocytes as a direct effect of binding.

Alefacept administration also leads to decreased expression of some genes in circulating PBMC (Fig. 3, Aii, Bi, and Biii). Several cell activation genes such as perforin (PRF1), killer cell lectin-like receptor B1 (KLRB1), CD69, RANTES, STAT4, and GZMB (Fig. 3, Aii and Bii) as well cell surface receptor genes including CD2, CD8A, IL-2RB (CD122), and IL-7R (Fig. 3Biii) were down-regulated at 6 h posttreatment. The reduction of CD8+ T cells by 53% (Fig. 1C) parallels the reduction of gene expressed produced by cytotoxic cells, such as GZMB (reduction by 70%, Fig. 3Aii). However, cytotoxic T cells as well as NK cells produce GZMB. Therefore, decreased CD8+ T cells, as well as reduction in NK cells, could explain the decreased expression of some “killer-associated” gene products.

### Table I. Alefacept preferentially and persistently decreases circulating CD8+ T cells

<table>
<thead>
<tr>
<th>Pretreatment vs</th>
<th>Mean Percent Decreased Number of CD8+ T Cells (95% CI)</th>
<th>Mean Percent Decreased Number of CD4+ T Cells (95% CI)</th>
<th>CD8+ vs CD4+ T Cells p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 h</td>
<td>49.64 (40.11–59.18)</td>
<td>31.26 (22.18–40.34)</td>
<td>0.0002</td>
</tr>
<tr>
<td>6 h</td>
<td>34.78 (11.70–57.86)</td>
<td>22.77 (2.76–42.77)</td>
<td>0.0503</td>
</tr>
<tr>
<td>24 h</td>
<td>42.36 (30.88–53.85)</td>
<td>27.42 (15.44–39.40)</td>
<td>0.0003</td>
</tr>
<tr>
<td>Week 2</td>
<td>49.64 (40.36–58.93)</td>
<td>31.26 (22.42–40.10)</td>
<td>0.0052</td>
</tr>
<tr>
<td>Week 4</td>
<td>36.49 (23.68–49.30)</td>
<td>20.33 (9.00–31.65)</td>
<td>0.0005</td>
</tr>
<tr>
<td>Week 13</td>
<td>32.93 (21.75–44.12)</td>
<td>18.15 (3.95–32.36)</td>
<td>0.0289</td>
</tr>
</tbody>
</table>

*p* Differences in effects of alefacept in CD4+ and CD8+ T cell populations. Mean percent decrease compared with pretreatment vs treatment with alefacept in vivo for 1 h, 6 h, 24 h, week 2, week 4, and week 13 with confidence intervals (CI) and *p* values for CD8+ vs CD4+ T cells are shown.

compared with baseline are indicated in green. The genes symbols, fold changes as ratio of posttreatment vs baseline, and relevant *p* values are also given. We confirmed expression differences of certain genes by real-time RT-PCR (Fig. 3B), which again showed that within 6 h of alefacept treatment of psoriasis patients, there was both an increase (Fig. 3Bii) and a decrease (Fig. 3B, ii and iii) in expression of different genes.

We have previously hypothesized that a “type 1” pathway might explain the pathogenesis of psoriasis (16). In this pathway, IL-12/IL-23 drives T cell activation, IFN-γ, and TNF induction, with subsequent increase in transcription factors STAT1 and NF-kB, leading to induction of downstream cytokines such as IL-8 and MIG. There are several genes of this “type 1” pathway that are up-regulated within 6 h, including IL-8, STAT1, and MIG (Fig. 3, Ai and Bi), which is opposite to what we would have predicted with this therapy (14). Gene expression data suggest that alefacept possibly could increase expression of inflammatory genes in leukocytes as a direct effect of binding.

Alefacept treatment rapidly modulates gene expression in healthy PBMC in vitro, but does not induce apoptosis

To confirm leukocyte activation in vivo, we analyzed the gene expression of healthy PBMC after 6 h exposure to alefacept in vitro. Approximately 1640 genes were differentially up- or down-regulated in PBMC treated with alefacept (Fig. 5A, supplemental Table Ib), with specific changes for selected genes shown in the list. Similar to in vivo exposure, we detected an increase in STAT1 gene expression in vitro. Alefacept treatment results in a relative lymphopenia, which could be due to apoptosis. We were not able to detect apoptosis of lymphocytes in vitro up to 72 h (Fig. 5B shows 6 h; 24 and 72 h data is not shown.), This implies that direct effects of alefacept on gene expression are independent of changes in cell numbers due to cell death.

Gene modulation by alefacept in vitro bears striking similarity to results of prior studies in which a large number of genes are activated or repressed after T cell stimulation by CD3 and CD28 Abs (19). To compare with alefacept-treated PBMC, we activated

### FIGURE 2. Rapid increase in gene expression of STAT1 and MIG in blood of patients treated with alefacept. Real-time RT-PCR analysis of expression of STAT1 and MIG (n = 3). Mean values of gene expression are graphically represented. mRNA was analyzed from blood samples at baseline (pre) and posttreatment. SEM, *, *p < 0.05.
T cells in PBMC preparations with CD3 and CD28 Abs and measured gene expression 6 h after Ab addition (supplemental Table Ic). There were both similarities and key differences in gene expression when comparing alefacept vs CD3/CD28-treated PBMC. We found 200 T cell activation-regulated genes, including STAT1, early growth response 2 (EGR2), and proliferating cell nuclear Ag (PCNA), were commonly modulated by CD3/CD28 Abs and by alefacept. There were two key differences in genes modulated by alefacept vs CD3/CD28 Abs. First, T cell activation by CD3/CD28 Abs led to selective, high-level induction of T cell effector cytokines such as IFN-γ and lymphotoxin (LTA) and it also led to strong up-regulation of mRNA for the IL-2 receptor-α-subunit (CD25), and these changes were not seen to such an extent in the alefacept-treated PBMC. Second, a significant set of genes was uniquely modulated by alefacept (see supplemental Table Ic). However, overall, these in vitro data support the concept that alefacept can directly activate gene expression in PBMC in vivo-treated patients.

From the analysis of genes modulated in PBMC in vitro by alefacept, it appears that alefacept alters gene expression in a way that is both agonistic and antagonistic for cell activation, survival, and production of inflammatory mediators. Fig. 5 and Table II list numerous genes that are regulated by alefacept treatment and where the biologic effects of gene products are proinflammatory, e.g., STAT1, IL-15, and MAPK activators (Table II). There are also effects mediated by up-regulation or down-regulation of specific genes that would be expected to decrease cell activation, survival, or inflammation (Table II). For example, some gene products that...
regulate cell growth in a negative fashion are induced by alefacept, while other genes that support T cell activation (TCR/β2m subunit and CD52) or inflammation (NF-κB) are down-regulated by alefacept. Therefore, we consider that in a biologic sense, alefacept might be best considered as a partial agonist or as a mixed agonist/antagonist.

Differences in responders and nonresponders before and after treatment with alefacept

Alefacept decreased circulating CD3+, CD4+, and CD8+ T cell subsets in responders (n = 9) to a larger extent compared with nonresponders (n = 8) (Fig. 6A). The mean depletion of CD8+ T cells is 57.7% as early as 1 h after administration of alefacept compared with 31.2% reduction of CD4+ T cells at this time point (p < 0.01 for both CD8+ and CD4+ T cells). The reductions in the T cell populations in nonresponders were variable.

We compared the gene expression changes in response to alefacept treatment in responders vs nonresponders using microarray and real-time RT-PCR analysis (Fig. 6B). Supplemental table Id describes all genes regulated at 6 h posttreatment in responders (supplemental Table IdA) and in nonresponders (supplemental Table IdB). There were a number of genes uniquely regulated in each group (supplemental Table IeA/B). Several genes of T cells or NK cell signaling were down-regulated in responders, including CD3D, CD2, and FoxP3 and (supplemental Table IeA). In nonresponders, TLR5 (Fig. 6B) and genes like spleen tyrosine kinase (SYK), known to induce expression of CD69, were up-regulated (supplemental Table IeB).

A subset of T cells that is of tremendous current interest is regulatory T cells, particularly in autoimmune diseases (20). We tested the gene expression of Foxp3, a known marker of regulatory T cells in circulating PBMC. At 6 h posttreatment with alefacept, only the responders showed an increased expression of this gene (p < 0.05, Fig. 6B). These data suggest that differential responses of patients to treatment with alefacept can be detected in blood as early as 6 h posttreatment.

As responders and nonresponders to alefacept have a different gene expression profile at 6 h in vivo, this led us to consider that there might be differences at baseline in these two groups. This background genomic heterogeneity might enable us to predict clinical outcomes without giving a trial period of alefacept treatment.

There were 199 genes that had significantly higher expression levels in pretreatment blood of responders compared with nonresponders (supplemental Table IeB). There were a number of genes uniquely regulated in each group (supplemental Table IeA/B). Several genes of T cells or NK cell signaling were down-regulated in responders, including CD3D, CD2, and FoxP3 and (supplemental Table IeA). In nonresponders, TLR5 (Fig. 6B) and genes like spleen tyrosine kinase (SYK), known to induce expression of CD69, were up-regulated (supplemental Table IeB).
levels in nonresponders vs responders (supplemental Table Ig). Several T cell-associated genes were expressed at higher levels in nonresponders vs responders including CD69, integrin, α6 (ITGA6), and CD3D (Fig. 6C and supplemental Table Ig). The expression of cell surface receptors known to be expressed on T cells and myeloid cells, such as CD2, CD8A, CD33 as well as TLR5 and Myd88 were higher in responders (Fig. 6C and supplemental Table Ig). Therefore, clear gene expression differences appear to categorize responders vs nonresponders to alefacept.

**Discussion**

Two potentially interrelated issues arise with the use of T cell-targeted biologics in the treatment of psoriasis: 1) only a subset of patients have high-grade improvement in skin diseases, as assessed by clinical or histologic criteria and 2) the in vivo mechanistic and immunologic effects of these drugs in patients are incompletely understood. Both problems are pertinent to the treatment of psoriasis patients with alefacept. The results of this study provide new insights into in vivo pharmacologic effects of alefacept in humans. There was strong and unexpected agonist activity for the induction of immune-related genes in vivo in circulating leukocytes that also manifests in the skin lesions. Type I genes like IL-8, STAT1, and MIG were up-regulated in PBMC, as well as IFN-γ and iNOS in skin. The set of genes, which is also increased by alefacept treatment in vitro, partly overlaps with genes induced by CD3/CD28 ligation in T cells. Therefore, immunologic effects in T cells may include induction of active transcriptional pathways.

Our observations suggest that alefacept has the potential to modulate immune responses through agonist or signaling effects on multiple cell types, which is very different view from its proposed actions as a CD2 antagonist or as an indirect lytic agent for effector T cells. Our results do not support the existing hypothesis for the mechanism of action of alefacept, namely that there is bridging between T cells and NK cells that leads to NK-mediated T cell apoptosis. We did not detect apoptosis in T cells in vivo or in vitro addition of alefacept to PBMC for periods up to 72 h. However, alefacept has been shown to activate gene expression in NK cells (21). Similar to their finding, we detected IL-15 as up-regulated after treatment of PBMC with alefacept. This suggests that the activation responses we measure could include activation of some NK genes and/or be dependent on bridging of cells via FcR plus CD2 co-ligation. We also acknowledge that cytokines released from cells lysed in vivo in accessible sites (for measurement) might also contribute to gene activation responses measured in vivo in alefacept-treated patients.

The chemokine lymphotactin (XCL2) is strongly up-regulated in peripheral blood leukocytes after treatment with alefacept in vitro (on average by ~6-fold, Fig. 5A), which raises a possibility that rapid reduction in circulating T cells after alefacept administration might be caused by chemokine-mediated retrafficking of T cells, rather than induction of apoptosis. We acknowledge as an alternative explanation that cell lysis occurring elsewhere might lead to release of cytokines or chemokines that alter cell-trafficking patterns.

A large set of immune-related genes were suppressed by in vitro incubation of leukocytes with alefacept. Decreased expressions of some genes, e.g., CD52 or TCR, suggest negative regulation of genomic pathways in T cells that could influence long-term activation or reactivation responses of T cells. Down-regulation of IL-7R by alefacept may also imply an inhibitory role of CD2 signaling in CD3-mediated differentiation and maturation of CD8+ T cells (22).

Alefacept may also affect expression of inflammatory genes in myeloid leukocytes. For example, p22^{max} (CYBA), a major protein in the respiratory burst pathway, known to be expressed by myeloid leukocytes (23) was detected to be decreased in PBMC treated with alefacept in vitro (Fig. 5B). As myeloid leukocytes usually express one or more FcR, ligation of FcR, particularly if re-enforced by cell bridging, may be mediating negative signals to FcR-containing cells. In psoriasis lesions, FcR-expressing myeloid...
cells like dendritic cells (DCs) are as abundant as T cells (17). Therefore, there is a possibility for direct immune modulation through FcR, including CD32b, which is known to be an inhibitory receptor for immune responses if activated (24).

Finally, the fact that alefacept induces meaningful disease improvement in only a subset of psoriasis patients has greatly limited its therapeutic application. Impressive disease improvement occurs in 20–50% of individuals treated with a standard 12-wk course of this agent, while other patients have little or no improvement in disease activity (14, 25). Prior work has not provided any clear explanation for this difference beyond the observation that skin-infiltrating T cells and DCs are consistently eliminated from psoriasis lesions in responding patients (14). No clinical or immune differences predict patients that will respond to alefacept, while our measures of alefacept binding to circulating T cells also reveal no differences between responders and nonresponders.

When obvious characteristics of divergent response groups are not evident, work with other therapeutics has shown that genomic or pharmacogenomic approaches have elucidated key response factors (26). We found differences between responders and nonresponders in two important areas: baseline gene sets and gene expression differences between responders and nonresponders could be induction of Foxp3 gene expression in the former group, but many other induced immune pathways could also contribute to this response difference. The induction of Foxp3 mRNA in responding patients suggests the potential for alefacept to stimulate regulatory T cells, which may help explain the mechanism of action of alefacept. Additional studies would be needed to pursue this finding in more detail.

Differences in baseline gene expression in responders and nonresponders may lead to the development of a “gene signature” that could predict psoriasis patients who are more likely to respond to alefacept therapy. Genes that are induced with a single dose of alefacept by 6 h could also be useful in this regard. There are many potential genes that might be used to make this distinction, but confirmation of these differences in a much larger clinical trial is now needed.

Disclosures
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References