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ORIGINAL ARTICLE

# Effect of Therapeutic Integrin (CD11a) Blockade with Efalizumab on Immune Responses to Model Antigens in Humans: Results of a Randomized, Single Blind Study

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Efalizumab is a humanized monoclonal CD11a antibody approved for treatment of psoriasis. Its immunomodulatory effects led us study how immune responses are modified and the possible consequences for vaccinations in clinical practice. This was a randomized, single-blind, placebo-controlled, parallel-group study of 12 weeks of subcutaneous efalizumab treatment of patients with moderate psoriasis. Bacteriophage ϕX174 was used as a model neoantigen to assess T-cell-dependent humoral immunity. Tetanus booster vaccine, pneumococcal vaccine, and intracutaneous skin tests were administered to further evaluate humoral and cellular immune responses. During efalizumab treatment, both primary and secondary antibody responses to ϕX174, including IgM/IgG isotype switch, were reduced. There appeared to be naïve T-cell anergy to a neoantigen (ϕX174) during active CD11a blockade, without tolerance to the antigen after efalizumab withdrawal. Secondary humoral immune responses to tetanus booster during treatment were reduced, but antibody titer increases led to protective levels. Responses to pneumococcal vaccination 6 weeks after withdrawal from efalizumab were not affected. Cellular immune responses to intracutaneous recall antigens were reduced during treatment and returned to pretreatment conditions after withdrawal. These results expand our knowledge of how immune responses are modulated in humans by CD11a blockade and have implications for vaccinations of patients treated with this agent.

*Journal of Investigative Dermatology* (2008) **128,** 2615–2624; doi:10.1038/jid.2008.98; published online 22 May 2008

## INTRODUCTION

The initial step in the activation of an immune response is presentation of an antigen-derived peptide bound to major histocompatibility complex on antigen-presenting cells to T cells. Interaction of costimulatory molecules expressed by antigen-presenting cells, such as CD80 (B7.1), CD86 (B7.2), CD40, or intercellular adhesion molecule-1, with their ligands, CD28, CD40L, or lymphocyte function-associated antigen-1 (LFA-1), is required for full T-cell activation. Efalizumab, a humanized mAb against CD11a, targets the α-chain subunit of the LFA-1 molecule and prevents its interaction with intercellular adhesion molecule-1. Efalizumab is approved in many countries for treatment of moderate-to-severe psoriasis, with safety and efficacy established in large phase-III trials (Gordon *et al.*, 2003; Lebwohl *et al.*, 2003). It is important to understand how therapeutic blockade of LFA-1 with efalizumab impacts the immune processes of autoimmune inflammation as well as normal, protective immune responses to foreign antigens.

In the initial phases of immune responses, binding of intercellular adhesion molecule-1 to LFA-1 plays an important role in forming the so-called immunological synapse between an activated antigen-presenting cell and a T cell, providing stabilization for signal exchange to achieve T-cell or B-cell activation, proliferation, and migration. LFA-1 blockade inhibits T-cell activation and natural killer cell cytolysis *in vitro* (Krensky *et al.*, 1983; Dustin and Springer, 1988) and T-cell-dependent B-cell proliferation and antibody production (Fischer *et al.*, 1986; Benjamin *et al.*, 1988), as well as target T-cell lysis and lymphocyte proliferation (Tanaka *et al.*, 1995). In addition to its function as a coactivator, LFA-1 blockade leads to reduced adhesion of T cells to the vascular endothelium (Dustin and Springer, 1988), resulting in reduced trafficking and homing of T cells into tissues (Hamann *et al.*, 1988; Issekutz, 1992). In preclinical studies with rodents, antibodies targeting CD11a-induced tolerance (anergy) toward donor

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*Abbreviations: CI, confidence interval; GM, geometric mean;* $K_v$*, K-value; LFA, lymphocyte function-associated antigen*

*Received 6 June 2007; revised 23 January 2008; accepted 27 January 2008; published online 22 May 2008*

antigens after bone marrow transplantation (Cavazzana-Calvo *et al.*, 1995) blocked the induction of experimental autoimmune encephalitis (Gordon *et al.*, 1995), reduced the incidence of corneal graft rejection (He *et al.*, 1994), and prolonged survival in various models of tissue and solid-organ allograft (Isobe *et al.*, 1992; Nakakura *et al.*, 1993; Talento *et al.*, 1993; Dedrick *et al.*, 2002). Although these anti-inflammatory and tolerance-inducing effects are desirable in the treatment of autoimmune diseases, they could also affect primary and secondary immune responses to foreign antigens and vaccinations.

In this study, we used several vaccines and model antigens to assess immune responses to proteins and polysaccharides. In general, acquired immune responses to polysaccharide antigens are dependent on intact B-cell function, whereas a full response to protein-based antigens also requires intact T-cell function. Because in theory primary presentation of a new protein antigen under certain circumstances, such as CD80/CD86 or anti-CD11a blockade, might induce permanent non-responsiveness (tolerance), a primary immune response should not be assessed with a human pathogen. Hence, the non-pathogenic, virus-like bacteriophage ϕX174 served as the protein neoantigen during and after efalizumab treatment. In normal individuals, ϕX174 triggers development of IgM antibodies to the first injection and T-cell-dependent switching to IgG antibodies usually starts with the second injection of ϕX174. In patients with inherited immune deficiency diseases, for example, severe combined immune deficiency, leukocyte adhesion deficiency disease (bearing a CD18 mutation), or X-linked agammaglobulinemia (as well as a number of other diseases), IgM or IgG antibody responses to ϕX174 are suppressed or absent (Ochs *et al.*, 1993; Price *et al.*, 1994). Therapeutic immune modulators such as CTLA4-Ig, which blocks T-cell costimulation (Abrams *et al.*, 1999), or rituximab, which depletes CD20+ B-cells (Bearden *et al.*, 2005), have been shown to suppress IgG antibody responses to ϕX174, with rituximab also suppressing the IgM response to this antigen (Bearden *et al.*, 2005). Hence, various aspects of immune function, such as antigen recognition, amplification, immunological memory, and isotype switching, can be well quantified in the ϕX174 system, whereas extensive prior testing of immune-altered individuals gives the ability to interpret outcomes within the context of the human immune system and immune deficiency (Gordon *et al.*, 2003; Lebwohl *et al.*, 2003). Using the ϕX174 antigen, we assessed the immunological recognition of a neoantigen by B cells and T cells during treatment, and addressed the questions, whether tolerance beyond treatment was induced in T cells via therapeutic LFA-1 blockade upon first antigen encounter. This study, with its wide spectrum of antigens used, provides key data on the mechanism of action of efalizumab in humans, as well as important clinical guidance for handling immunizations during its use.

## RESULTS

### Patient disposition

Sixty-six patients with moderate plaque psoriasis were enrolled. Of the 66 patients enrolled and randomized, 62 (94%) completed the 12-week treatment period and 61 (92%) completed the 14-week follow-up (Figure 1). Six patients randomized to group B unintentionally received active ϕX174 vaccine during the efalizumab treatment period; in all analyses, these patients are included *as treated* in group A. Thus, 28 patients were included in group A, 16 in group B, and 22 in group C. Demographic and baseline psoriasis characteristics were generally comparable across treatment groups (Table 1). The study design is shown in Figure 2a. Groups A and B received Efalizumab (1 mg/kg) weekly for 12 weeks, Group C received placebo treatment. For immunizations, Groups A and C received the experimental antigen ϕX174 twice *during* the first 12 weeks and twice *after* study drug discontinuation. Group B received vehicle ϕX174 twice *during* the treatment period and ϕX174 twice *after* the treatment period. Tetanus booster vaccinations, intracutaneous skin tests and pneumococcal vaccines were administered as shown in the study design (Figure 2a).

### Pharmacodynamic effect of efalizumab on CD11a-binding sites and expression

During efalizumab treatment, all CD11a-binding sites on circulating B and T lymphocytes were occupied 100% by efalizumab (data not shown). In addition, the number of remaining CD11a molecules on the surface of T cells was reduced by approximately 80% for both groups A and B (and this reduction or down-modulation was maximal by day 35), as assessed with a CD11a antibody binding to a distinct epitope from efalizumb (Figure 2b). Efalizumab is eliminated from the body approximately 7 weeks after the last dose (follow-up day 42) (Gottlieb *et al.*, 2000). By day 42 of the efalizumab wash-out follow-up period, the number of CD11a molecules on the surface of T cell returned to normal (Figure 2b) and no residual efalizumab binding could be detected (data not shown).

As expected, CD11a expression remained close to baseline throughout the study for group C (Figure 2b). During active efalizumab treatment, CD11a expression on B cells was downregulated by approximately 35–40% of baseline (Figure 2c).

### Antibody responses to ϕX174

During treatment, antibody responses to ϕX174, measured as phage-neutralizing activity and expressed as *K*-value ($K_v$), were determined at immunization and 2 and 4 weeks following immunization (Figure 3a). The geometric mean (GM) $K_v$ was approximately 10-fold lower in the efalizumab-treated group A, compared with that in placebo-treated group C, and was also below the lower 95% confidence interval (CI) determined for group C. However, substantial antibody responses occurred in efalizumab-treated patients (group A) and the resulting antibody titer was sufficient to neutralize ϕX174 after the second and subsequent immunizations (no circulating infectious phage was present in the peripheral blood 15 minutes after these infusions). These results indicate B cells recognize ϕX174 antigen during efalizumab treatment and that recall responses and immune amplification are largely intact. Antibody responses to immunizations were

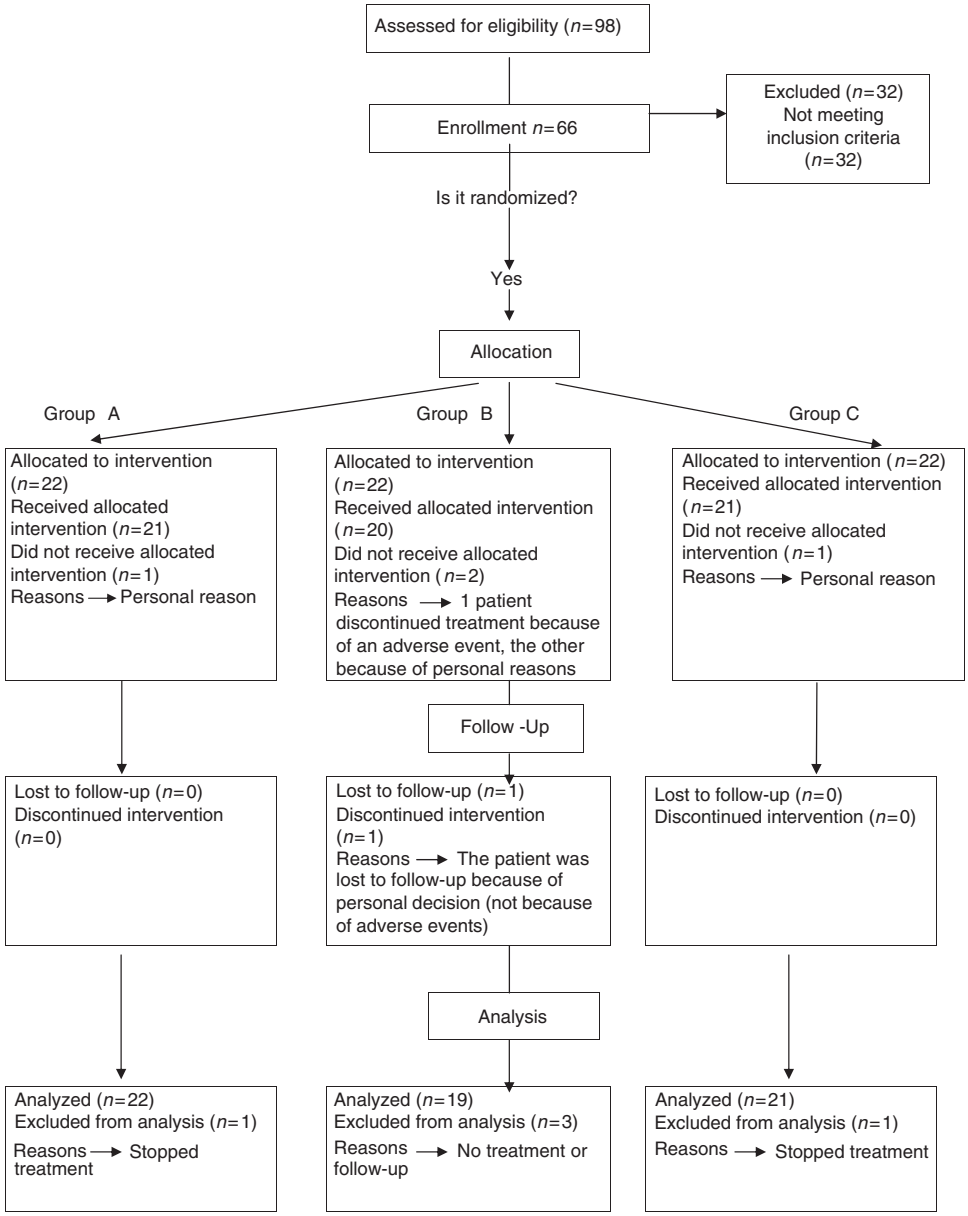



**Figure 1. The CONSORT flowchart.**

further characterized by determining the isotype switching from IgM to IgG, which is dependent on T-cell recognition of a protein-based antigen. At 2 weeks post secondary immunization, 0.13% of the antibody was of the IgG isotype in groups A and B *versus* 25% in group C (Figure 3b). Hence, anti-ϕX174 antibodies failed to isotype switch during efalizumab treatment, indicating a likely state of T-cell anergy to ϕX174. However, when boosted with phage (immunizations 3 and 4) after efalizumab discontinuation, both group A and group B patients had an increase in antibody titer with 8 and 15% IgG antibody, respectively, at 2 weeks and 11 and 20% at 4 weeks after the fourth immunization. This indicates T-cell responsiveness to ϕX174 upon efalizumab wash out, such that permanent T-cell anergy and tolerance to this phage were not induced, although magnitude of the antibody response was still somewhat reduced.

Immunization with ϕX174 after discontinuation of efalizumab in patients who had received phage vehicle during efalizumab treatment (group B) resulted in an antibody response slightly lower in magnitude compared with the first two immunizations in group C patients (Figure 3a), suggesting some residual efalizumab effect at follow-up day 42. The reduced response was independent of CD11a expression (Figure 2b and c) or saturation of CD11a-binding sites on T cells or B cells.

### Antibody responses to tetanus immunization

Four weeks after tetanus booster immunization given on treatment day 35, efalizumab-treated patients (groups A and B)

**Table 1. Baseline demographic and psoriasis characteristics**

| Characteristic | Group A (efalizumab+ ϕX174) (*n*=28) | Group B (efalizumab+ vehicle immunization) (*n*=16) | Group C (placebo+ ϕX174) (*n*=22) |
|---|---|---|---|
| *Age (years)* | | | |
| Mean (SD) | 45.0 (11.6) | 40.9 (12.1) | 40.4 (9.9) |
| Median (range) | 45 (21–65) | 38 (19–63) | 42 (23–58) |
| *Sex, n (%)* | | | |
| Men | 23 (82.1) | 9 (56.3) | 13 (59.1) |
| Women | 5 (17.9) | 7 (43.8) | 9 (40.9) |
| *Race/ethnicity, n (%)* | | | |
| White | 25 (89.3) | 13 (81.3) | 18 (81.8) |
| Black | 0 | 2 (12.5) | 0 |
| Asian/ Pacific Islander | 1 (3.6) | 1 (6.3) | 2 (9.1) |
| Hispanic | 1 (3.6) | 0 | 0 |
| Other | 1 (3.6) | 0 | 2 (9.1) |
| *Duration of psoriasis (years)* | | | |
| Mean (SD) | 15.2 (9.2) | 17.5 (8.7) | 14.1 (9.1) |
| Median (range) | 13.5 (1–32) | 15.5 (4–39) | 11.5 (4–39) |
| *BSA affected (%)* | | | |
| Mean (SD) | 11.3 (2.2) | 11.7 (2.1) | 11.2 (2.3) |
| Median (range) | 11.1 (8.0–14.5) | 11.3 (8.0–14.5) | 10.5 (8.0–15.0) |
| *Prior systemic or phototherapy for psoriasis, n (%)* | | | |
| Yes | 11 (39.3) | 10 (62.5) | 9 (40.9) |
| No | 17 (60.7) | 6 (37.5) | 13 (59.1) |

BSA, body surface area.

showed an approximately threefold increase in the levels of anti-tetanus toxoid IgG antibody (Figure 4a). The increase in placebo-treated group C was approximately 10-fold. At screening, 31 of 43 (72%) efalizumab-treated patients and 17 of 22 (77%) placebo-treated patients had anti-tetanus toxoid IgG levels $\geqslant 0.5\,\mathrm{IU\,ml^{-1}}$; 4 weeks after tetanus immunization, 40 of 42 (95%) efalizumab-treated patients and all of 22 (100%) placebo-treated recipients had positive anti-tetanus toxoid IgG levels (Figure 4b). In addition, 35 of 42 (83%) of efalizumab-treated patients and 21 of 22 (95%) of placebo-treated recipients demonstrated an increase in anti-tetanus toxoid IgG levels of $\geqslant 0.5\,\mathrm{IU\,ml^{-1}}$ above preimmunization levels at 4 weeks after tetanus immunization.

### Antibody responses to pneumococcal polysaccharides

The assay used in this study detects IgG antibodies against 12 of the 23 antigens included in Pneumovax®. At baseline, before immunization patients in all groups generally had antibody titers $\geqslant 1.4\,\mu\mathrm{g\,ml^{-1}}$ IgG to at least some pneumococcal antigens (typically one to three strains). The increase in the number of positive titers after vaccination to any of the 12 measured pneumococcal antigens was determined and compared in the efalizumab- and placebo-treated groups. The percentage of patients who converted antibody levels to at least one ($\geqslant 1$) and $\geqslant 2$, $\geqslant 3$, and $\geqslant 6$ additional pneumococcal strains was generally similar between the efalizumab and placebo groups (Figure 5). Increases in median antibody levels after immunization were also similar between groups (data not shown).

### Skin test responses to recall antigens

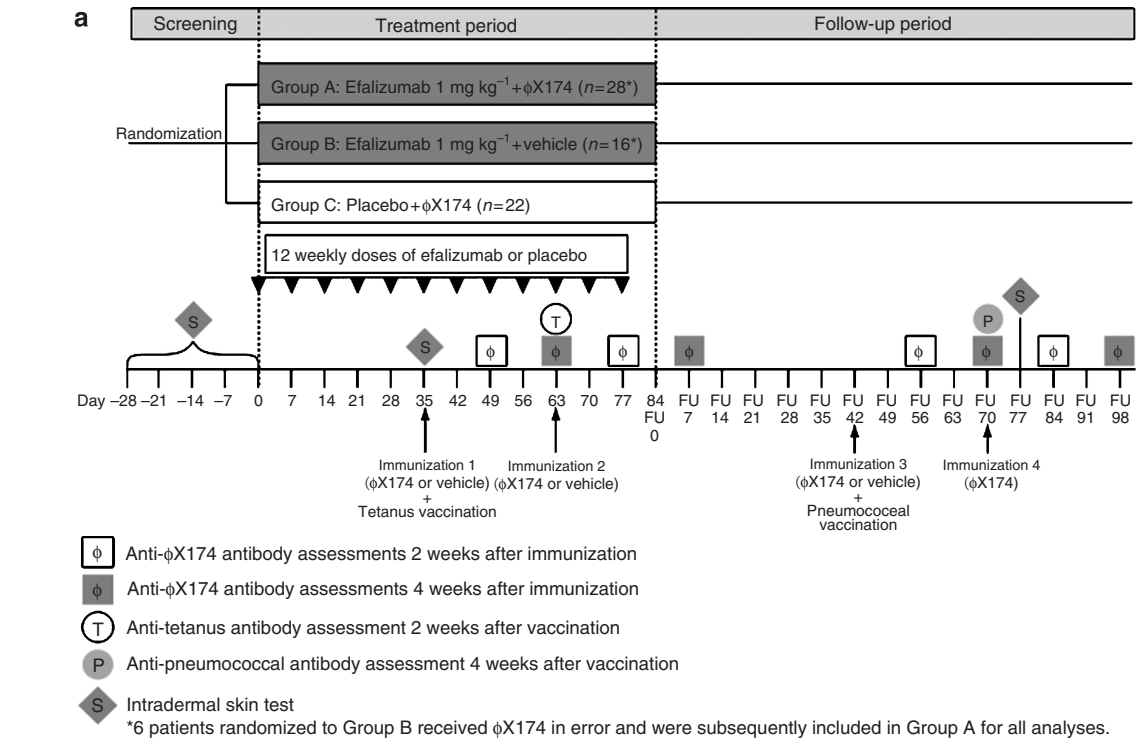
At screening, approximately 80% of all patients had at least one positive skin test (Figure 6). Skin test results at day 35 were positive to *Candida albicans* in 57 and 91% of efalizumab- and placebo-treated patients, respectively ($P=0.009$). By follow-up day 77, skin tests were similar to baseline in all the groups (Figure 6a). At screening, the percentage of patients with a positive tetanus toxoid skin test reaction was low for all the groups: 52% for patients to be treated with efalizumab *versus* 36% for patients to be treated with placebo (Figure 6b). The proportion of efalizumab-treated patients with positive skin test reactions to tetanus toxoid skin testing was reduced compared with placebo-treated patients at day 35 (24 *vs* 41%) and increased only slightly by follow-up day 77 (30 *vs* 38%).

### Adverse events experienced during the study

The most frequent adverse events were infection (32% for placebo-treated patients *versus* 16% for efalizumab-treated patients). One serious adverse event occurred in a placebo-treated patient. The most frequent adverse events for efalizumab-treated patients were psoriasis (52%) and headaches (39%), mostly mild to moderate; there was one severe exacerbation of psoriasis. No adverse events of arthritis, hemolytic anemia, or thrombocytopenia were reported. No adverse events were associated with immunizations.

## DISCUSSION

Therapeutic intervention for autoimmune diseases seeks to dampen pathological immune responses toward self-antigens. However, in doing so, immune responses to pathogens or vaccines might also be affected in an undesired manner. There is little information on how novel biological treatments impact immune responses, for example, to vaccines, despite this being an important health issue. Moreover, because animal models demonstrated tolerance induction through blockade of costimulatory molecules on T cells and CD11a specifically (Benjamin *et al.*, 1988; Cavazzana-Calvo *et al.*, 1995), a theoretical concern could be the induction of sustained unresponsiveness to viral or other pathogens in humans. Thus, thorough characterization of immune responses to antigens during and after any immunomodulatory



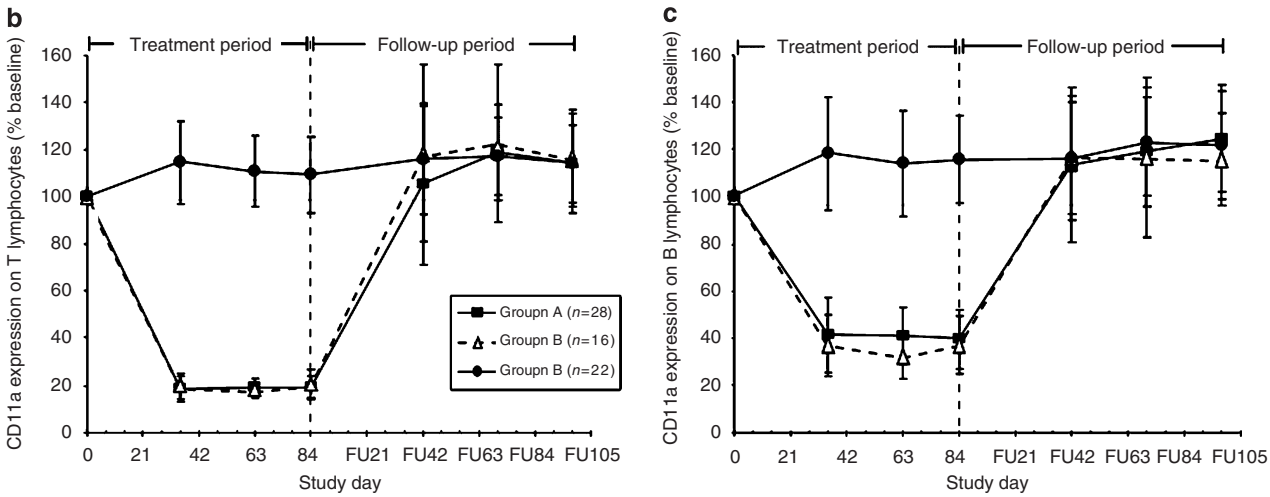




**Figure 2. Study design and rationale for immunization time points.** (**a**) The study schema: In the treatment period (day 0 through day 84 (=FU day 0)) patients received 1 mg kg$^{-1}$ week$^{-1}$ of efalizumab (groups A and B) or placebo (group C). All patients underwent a 14-week follow-up period (FU days 0–98). Group A and group C (placebo) patients were immunized with ϕX174 twice during (days 35 and 63 of efalizumab therapy) and twice after efalizumab (day 42 and day 70 after efalizumab was discontinued) treatment; group B patients were immunized with ϕX174 twice during FU only (days 42 and 70 of follow up). Additional experimental immunizations were conducted in all patients shown at the indicated time points. (**b**) Mean CD11a expression (with SD) on CD3+ T lymphocytes demonstrates maximum downregulation by the time of first immunization and recovery by the time of FU immunizations. (**c**) Mean CD11a expression on B cells and downregulation to approximately 40% of baseline by day 35 with recovery by FU day 42. FY, follow-up.

treatment is of major clinical relevance. This trial used a comprehensive set of model antigens and vaccines to investigate humoral and cellular responses, and addressed the question of induction of sustained unresponsiveness to a model antigen in humans.

The results of this trial further define the effect of efalizumab on primary and recall immune responses in psoriasis patients treated with therapeutically relevant doses of the CD11a antibody. In untreated control patients, the primary B-cell response to bacteriophage ϕX174 generated high levels of IgM antibodies, whereas efalizumab-treated patients demonstrated a lower, but still substantial response to ϕX174. Overall, the levels of antibody attained after the first antigen exposure were maintained in control patients as well as in efalizumab-treated patients. Immunological memory to the ϕX174 antigen seems to be apparent in both control and efalizumab-treated patients, suggested by further increases in anti-ϕX174 antibody levels upon second, third,

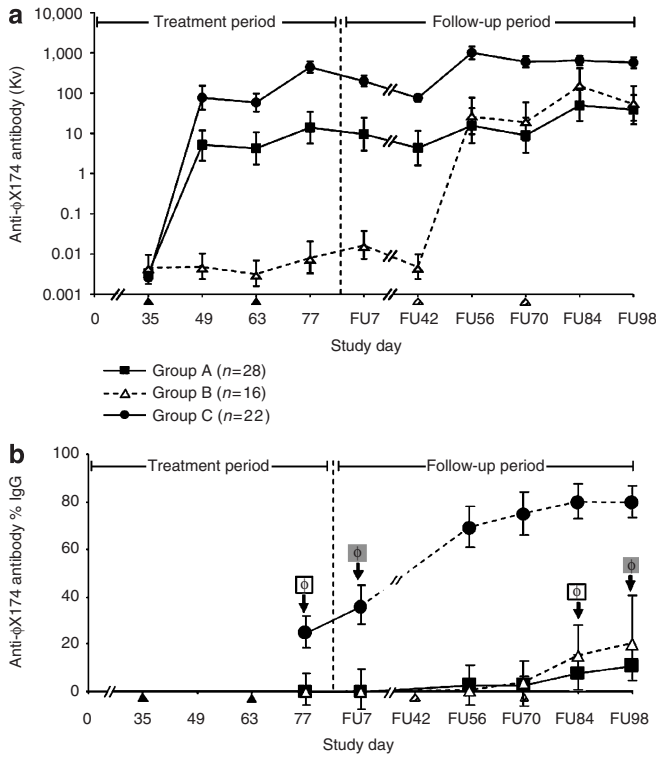



**Figure 3. Antibody response to ϕX174.** (**a**) GMs of total anti-ϕX174 phage-inactivating activity ($K_v$) with 95% CIs at each data point. ▲: Immunization with ϕX174 (groups A and C) or vehicle (group B) and ◬: immunization with ϕX174 (group B, immunizations 1 and 2; groups A and C, immunizations 3 and 4). (**b**) IgM to IgG isotype switch upon re-immunization. GMs of percent IgG of anti-ϕX174 with 95% CI shown at each data point. ▲: Immunization with ϕX174 (groups A and C) or vehicle (group B); ◬: immunization with ϕX174 (group B); ϕ: anti-ϕX174 antibody assessments 2 weeks after immunization 2 or 4; and ϕ: anti-ϕX174 antibody assessments 4 weeks after immunization 2 or 4.

and fourth injections of this bacteriophage (groups A and C). Psoriasis patients treated with efalizumab and exposed to ϕX174 during the active treatment phase (group A) showed blockade of the IgM-to-IgG isotype switch, indicating an impaired naïve T-cell response to this protein-based neoantigen. However, upon discontinuation of efalizumab and re-expression of LFA-1 on T cells, subsequent exposure to ϕX174 produced substantial levels of IgG antibodies (group A), indicating return of T-cell helper function. Subjects treated with efalizumab but receiving ϕX174 for the first time in follow-up (group B) displayed both IgM and IgG antibody responses. Isotype switching in both efalizumab-treated groups was still somewhat reduced as compared with that in control patients (group C), but reflected mostly competent T-cell function. Hence, during active LFA-1 blockade, naïve CD4+ T cells appear to be in an anergic state, with impaired ability to provide T-cell help to B cells, but tolerance beyond cessation of efalizumab treatment is not induced. Finally, immunizations with ϕX174 led to a degree of antibody production (IgM or IgG) sufficient to rapidly clear circulating ϕX174 15 minutes after injection. Quantitative levels of IgM or IgG likely determine the duration and effectiveness of immunity against this antigen.

Responses to a tetanus booster vaccination during (a protein antigen), and to pneumococcal antigens (carbohydrate antigens) after efalizumab treatment, are present, although the magnitude of tetanus response was lower than that in in control patients. Delayed-type hypersensitivity responses, which are dependent on antigen presentation as well as cellular trafficking to the skin, are both only moderately suppressed during efalizumab treatment, which is surprising as theoretically LFA-1 blockade could prevent migration of memory T cells into the skin at an inflammatory site.

The results obtained in this study revealed less impact on immune responses than in other situations of immunomodulation. Immunization data from rituximab, an anti-CD20 B-cell-depleting antibody, led to stronger ablation of antibody responses to ϕX174 (Bearden *et al.*, 2005). Moreover, in patients with primary or acquired T-cell immune deficiency, reduction of antibody responses to ϕX174 was much stronger than during efalizumab treatment (Ochs *et al.*, 1971, 1993; Pyun *et al.*, 1989; Price *et al.*, 1994). Finally, the relatively rapid re-expression of CD11a on T cells after discontinuation of efalizumab treatment suggests quick reversibility of effects.

The data reported here are consistent with those of another recent study showing that efalizumab treatment strongly reduced T-cell activation produced by a polyclonal stimulus (T-cell activation by CD3 ligation), with this effect being fully reversible upon wash out of efalizumab (Guttman-Yassky *et al.*, 2007). Overall, T-cell reactivity and reduced expression of T-cell-surface proteins were reduced only when efalizumab was present at saturating levels for CD11a, and these effects are reversed fully upon discontinuation of treatment (Guttman-Yassky *et al.*, 2007).

Results presented here may be considered in the clinical management of vaccinations for psoriasis patients undergoing treatment with efalizumab, although it is generally unclear whether or not any result using a model vaccination antigen can be extrapolated to other test systems or clinically used vaccination. Moreover, the role of protective antibodies, which can be measured relatively easily in a clinical trial setting, *versus* the role of antigen-specific T-cell memory is not fully understood. Opinions range from the view that antibodies are primarily responsible for the control of bacterial infections and are dispensable for the control of most viral infections (Whitten and Oldstone, 2001), to the view that antibodies are the only identified agents of successful vaccine protection (Zinkernagel *et al.*, 2001). Thus, the fact that administration of neoantigen during treatment led to an IgM response, but a block in IgG/IgM isotype switch, suggests that some immunity during treatment is likely still induced, but may not be as high and long-lasting as a full blown response in an untreated control population. Therefore, live vaccines are not to be recommended during efalizumab treatment, although this study did not directly address this situation. Since withdrawal of the immunomodulatory agent widely re-installed IgG isotype switch, no detrimental long-term effect beyond drug withdrawal is anticipated for killed vaccines. Tetanus booster immunization during treatment led to a meaningful increase of anti-tetanus

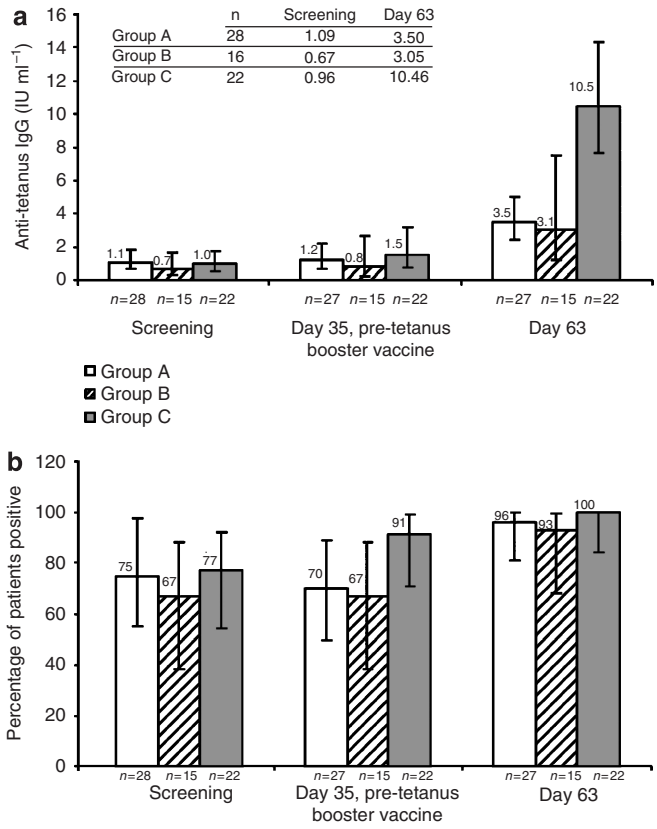



**Figure 4. Response to tetanus booster vaccine.** (**a**) GMs of anti-tetanus toxoid (IgG titers (IU ml$^{-1}$) before and 4 weeks after booster vaccination on day 35 during treatment period, with 95% CIs shown at each data point. (**b**) Percentage of patients with anti-tetanus toxoid IgG $\geqslant$0.5 IU ml$^{-1}$ (with 95% CIs based on the binomial distribution) at each data point. Positive anti-tetanus antibody levels defined as $\geqslant$0.5 IU ml$^{-1}$.

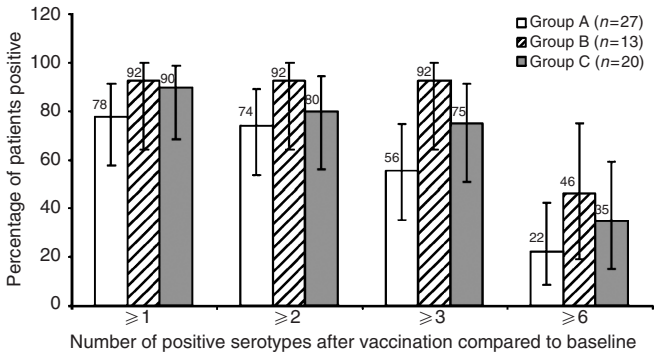



**Figure 5. Response to pneumococcal vaccine antigens.** Percentage of patients (with 95% CIs based on the binomial distribution at each data point) with positive antibody responses to one or more pneumococcal antigens compared with the baseline 4 weeks after Pneumovax 23 vaccination administered on FU day 42. FU, follow-up.

IgG and is likely to enhance immunity even during efalizumab treatment. Finally, pneumococcal vaccinations were not impacted at all after withdrawal from efalizumab. These conclusions are similar to those given for other immune modulators such as prednisone, metrotrexate,

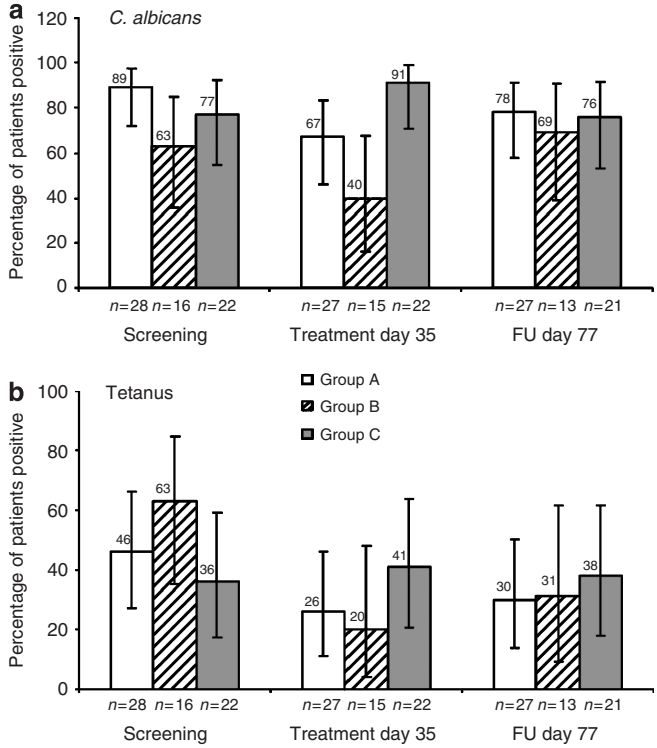



**Figure 6. T-cell-mediated skin test response to recall antigens before, during, and after efalizumab treatment (with 95% CIs based on the binomial distribution at each data point).** (**a**) Response to *C. albicans* antigens. (**b**) Response to intracutaneous tetanus toxoid antigen.

cyclosporine A, mycophenolate mofetil, etanercept, and others (Derkx *et al.*, 1993; Elliott *et al.*, 1994a, b, 1997; van Dullemen *et al.*, 1995; Moreland *et al.*, 1996, 1997; Dengler *et al.*, 1998; Abrams *et al.*, 1999; Mease *et al.*, 2000; Van den Bosch *et al.*, 2000; Chaudhari *et al.*, 2001; Ellis and Krueger, 2001; Ogilvie *et al.*, 2001). More data on the role of CD11a blockade with regards to infections are accumulating. Of interest is a recently published pooled analysis of phase-III clinical trials, indicating that the incidence and severity of infections in efalizumab- and placebo-treated patients were similar during the controlled portion of trials, and the incidence of infection did not increase with extended therapy up to 27 months (Langley *et al.*, 2005).

This study also demonstrates that the impact of immune modulators may be different in humans from what is predicted by gene-knockout or therapeutic surrogate animal models of immunity and disease. Although operationally and technically extremely challenging, more human immunization data will further help us understand the entire impact of biological and non-biological immunotherapy in humans (Derkx *et al.*, 1993; Bohmig *et al.*, 1994; Elliott *et al.*, 1994a, b, 1997; van Dullemen *et al.*, 1995; Moreland *et al.*, 1996, 1997; Dengler *et al.*, 1998; Abrams *et al.*, 1999; Mease *et al.*, 2000; Van den Bosch *et al.*, 2000; Chaudhari *et al.*, 2001; Ellis and Krueger, 2001; Ogilvie *et al.*, 2001; Whitten and Oldstone, 2001; Zinkernagel *et al.*, 2001; Langley *et al.*, 2005).

## MATERIALS AND METHODS

### Study design

Genentech Protocol ACD2244g, Protocol Amendment I, and the Subject Informed Consent for this single-site study were submitted to, reviewed by, and approved by IRB Services (Aurora, ON, Canada). All subsequent amendments and versions of the patient consent were similarly Institutional Review Board-approved for this study. All patients enrolled in ACD2244g gave consent for participation in the study via the Institutional Review Board-approved consent form prior to commencement of any study procedures. This study is registered at clinicaltrials.gov. Its unique identifier/registration number is NCT00382512. The study was conducted according to the Declaration of Helsinki Principles.

Ninety-eight patients were screened for this study, and 66 with moderate plaque psoriasis (8–15% affected body surface area, $n=66$) between 18–65 years old, with no evidence of infection, malignancy, pregnancy, and history of hypersensitivity reaction to any of the administered vaccinations, were randomized and enrolled in the study. Of the 66 patients randomized, four patients discontinued study drug during the treatment period: 1 (3.6%) in group A, 2 (12.5%) in group B, and 1 (4.5%) in group C. One patient in group B (6.3%) discontinued the drug during the follow-up period (Figure 1). The eligible patients were randomized using a computer-generated schedule in blocks of six individuals into three experimental groups (A–C), and allotted the next sequential number on the randomization schedule, which was predetermined and associated with a specific treatment. All patients did not receive systemic or ultraviolet therapy for at least 4 weeks prior to screening day. Topical treatment was allowed on untested skin. McDougall Scientific Limited (Toronto, ON, Canada) was responsible for generation of the randomization schedule. Allied Clinical Research was responsible for conduct of the study. This was a single-blind study. A trained research-team member administered all doses of the study drug. Patients were analyzed according to their actual study treatments and ϕX174 immunizations.

Groups A and B received 1 mg $kg^{-1}$ efalizumab treatment weekly for 12 weeks and were followed thereafter for an additional 14 weeks (''wash out''). Group C received placebo for 12 weeks and was followed for an additional 14 weeks. The main study objective was to measure primary and secondary immune responses to the experimental antigen ϕX174 administered during and after efalizumab treatment (group A), *versus* ϕX174 administered only after efalizumab treatment (group B). The study also had the secondary objective of measuring immune responses to several unrelated protein or polysaccharide antigens using two parenteral antigens (tetanus toxoid and pneumococcal vaccine) given systemically and two skin test antigens (*C. albicans* and tetanus toxoid).

All patients were monitored for safety through the 14-week follow-up period. The Psoriasis Area and Severity Index scores (efficacy) were not measured in this study. A placebo group (group C) was included to establish baseline immune responses in psoriasis patients (study design; Figure 2a). The determination of sample size was based on a non-inferiority test using the hypothesis that the mean anti-ϕX174 antibody concentration of each of the efalizumab groups would be no less than two SDs below the mean for the placebo group.

Binding of efalizumab treatment to CD11a on B cells and T cells was assessed by flow cytometry using mAb MHM24 (Dako Corporation, Carpinteria, CA), which binds to the same epitope on CD11a as efalizumab. Modulation (downregulation) of CD11a expression on lymphocytes was determined using mAb HI111 (BD Pharmingen, San Diego, CA), which binds to an epitope on CD11a different from efalizumab.

### Immunization with ϕX174

ϕX174 ($1\times10^{11}$ plaque-forming units per ml) or vehicle was administered as an intravenous bolus at a dose of 0.02 ml $kg^{-1}$ body weight ($2\times10^{9}$ plaque-forming units per kg) on day 35, day 63, and on follow-up days 42 and 70. Blood samples to detect circulating ϕX174 were collected 15 minutes after phage injection. Sera for antibody determination were obtained 2 and 4 weeks after each administration of ϕX174 or vehicle. Antibody titers are expressed as the rate of phage inactivation, or $K_v$, as described previously (Ochs *et al.*, 1971). The phage-neutralizing antibody resistant to treatment with 2-mercaptoethanol is considered as IgG (Ochs *et al.*, 1971; Pyun *et al.*, 1989). The mean percent IgG and CI were calculated on the arcs in transformed values and then transformed back to the percent scale for reporting.

### Immunization with tetanus toxoid

All patients received an intramuscular tetanus booster vaccination (Td absorbed™; Aventis Pasteur Limited, Toronto, ON, Canada) on day 35. Antibody levels were measured by IBT Reference Laboratory (Lenexa, KA) using an enzyme immunoassay that has a dynamic range of 0.01 to 18.5 IU $ml^{-1}$ IgG and a detection limit of 0.01 IU $ml^{-1}$. Anti-tetanus toxoid IgG antibody levels $\geqslant$0.5 IU $ml^{-1}$ were defined as positive and protective. An increase in anti-tetanus toxoid IgG $\geqslant$0.5 IU $ml^{-1}$ above baseline is classified as a ''clinically meaningful'' increase.

### Immunization with pneumococcal polysaccharides

A pneumococcal vaccine containing 23 pneumococcal polysaccharide types (Pneumovax 23 pneumococcal vaccine polyvalent; Merck & Co. Inc., Whitehouse Station, NJ) was used (Figure 2). A fluoroimmunoassay was developed and performed by IBT Reference Laboratory. Serum IgG antibody concentrations against 12 of the 23 pneumococcal antigens contained in the vaccine were determined (US nomenclature strains 1, 3, 4, 8, 9, 12, 14, 19, 23, 26, 51, and 56). The detection limit for antibodies against each strain was 1.4 μg $ml^{-1}$. As many patients in this study had measurable anti-pneumococcal antibodies against some strains (typically 1–3) at baseline, antibody responses to each of the 12 antigens were determined after immunization and compared with the baseline; the difference in the number of antigens recognized is shown for all pooled efalizumab patients and compared with the placebo group. Median changes in individual antibody titers were also determined.

### Skin testing for delayed-type hypersensitivity

Recall antigens *C. albicans* (Candin; Allermed Laboratories Inc., San Diego, CA) and tetanus toxoid (1:10 diluted; Aventis Pasteur Inc., Swiftwater, PA) were administered intracutaneously on the patient's forearm and observed 48–72 hours after application. A positive reaction was defined as induration and erythema $\geqslant$2 mm.

### Statistical analysis and evaluations

Data management and statistical analysis activities for the study were conducted under the overall responsibility of Janet McDougall

at McDougall Scientific Ltd. The analysis intent-to-treat population for vaccination endpoints consisted of all patients who were randomized into the study and had received any amount of the study drug. Endpoints included quantification of the antibody responses to ϕX174 ($K_v$) at various time points, isotype switch (%IgG) following each booster immunization with ϕX174, anti-tetanus toxoid antibody titers (IgG), anti-pneumococcal antibody titers to 12 serotypes, and intracutaneous skin test reactions to tetanus and *Candida* antigens (delayed-type hypersensitivity). The GM of the anti-ϕX174 antibody titers, expressed as $K_v$ and determined 2 weeks after immunizations 3 and 4 for group A and immunizations 1 and 2 for group B, were compared with the GM *K*-values observed in group C. A one-way analysis of variance was performed at each time point using a model with treatment group as the independent factor and Dunnett's adjustment to construct simultaneous 95% CIs for the difference between mean $\log_{10}$ anti-ϕX174 antibody $K_v$ for each efalizumab-treated group and the placebo-treated group.

## CONFLICT OF INTEREST

EG was and WD is an employee at Genentech Inc.

## ACKNOWLEDGMENTS

We thank Dr Flavius Martin, Department of Immunology, Genentech Inc., for critical reading and valuable input into the paper. Robert Quesenberry (Genentech) and Dunya Botetzayas (Allied Clinical Research) deserve much credit for expert clinical management of this trial. We appreciate the editorial contributions of Patricia Segarini, PhD, toward preparation of the paper.

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