

Immediate Type I Hypersensitivity Response Implicated in Worsening Injection Site Reactions to Adalimumab

Michael Paltiel, MD; Laura M. Gober, MD; April Deng, MD, PhD; Jamal Mikdashi, MD; Irena Alexeeva, MD; Sarbjit S. Saini, MD; Anthony A. Gaspari, MD

Background: Tumor necrosis factor (TNF) inhibitors such as adalimumab, etanercept, and infliximab play an increasingly important role in the management of a variety of chronic inflammatory disorders. With their increasing use, a wide spectrum of dermatological adverse effects, including injection site reactions and the development of dermatitis, have been recognized. Previous studies have implicated the role of the delayed-type hypersensitivity reaction in mediation of injection site reactions to etanercept. To our knowledge, there have been no published studies on immunologic mechanism of injection site reactions to adalimumab to date.

Observations: We describe 2 patients with a history of worsening injection site reactions to adalimumab. Findings from skin testing in both patients were suggestive of

an immediate type I hypersensitivity reaction to adalimumab. A histamine release assay performed on peripheral blood leukocytes from both patients demonstrated significant histamine release on exposure to adalimumab. Furthermore, passive transfer of serum from one of the allergic patients to basophils from a nonatopic, healthy donor sensitized those cells to release significant amounts of histamine with exposure to adalimumab.

Conclusion: This study demonstrates that an IgE-mediated immediate type I hypersensitivity reaction plays a role in the mediation of worsening injection site reactions in some patients receiving adalimumab.

Arch Dermatol. 2008;144(9):1190-1194

THE ADVENT OF TUMOR necrosis factor (TNF) inhibitors has dramatically changed the therapeutic approach to an increasing number of immune-mediated inflammatory disorders including psoriasis and psoriatic arthritis,¹ rheumatoid arthritis,²⁻⁴ juvenile idiopathic arthritis,⁵ ankylosing spondylitis,^{6,7} ulcerative colitis,⁸ and Crohn disease.⁹ Currently available anti-TNF agents include 1 soluble p75 TNF receptor (etanercept) and 2 monoclonal anti-TNF antibodies (infliximab and adalimumab). While generally well tolerated and safe, a variety of adverse effects including a wide spectrum of dermatological conditions are increasingly being recognized in the setting of TNF inhibitors.

In clinical trials, a variety of skin conditions have been reported, including cutaneous eruptions and injection site reactions (ISRs) during adalimumab therapy,^{4,10} urticarial reactions and stomatitis during infliximab therapy,¹¹ and ISRs during etanercept therapy.³ Zeltser et al¹² conducted a retrospective study of patients who experienced etanercept-induced ISRs by performing an immunohistological analysis of reaction sites in 3 patients with rheumatoid arthritis. The authors observed an inflammatory infiltrate composed largely of T cells bearing an activated cytotoxic phenotype (HLA-DR⁺/CD3⁺/CD4⁻/CD8⁺), with a minor contribution of cells bearing an activated helper/inducer T-cell phenotype (HLA-DR⁺/CD3⁺/CD4⁺/CD8⁻), CD14⁺ monocytes, CD1⁺ Langerhan cells, eosinophils, and neutrophils. The authors speculated that their findings were most consistent with a T-cell-mediated delayed hypersensitivity reaction, mediated by CD8⁺ T cells.¹² As with etanercept, patients receiving adalimumab therapy commonly experience ISRs. To our knowledge, however, no studies have been conducted to assess the immunologic mechanism of ISRs with adalimumab therapy to date. We describe 2 patients with prominent and worsening ISRs to adalimumab and present evidence demonstrating that immediate-type hypersensitivity reaction plays a role in the mediation of ISRs in both patients.

Author Affiliations:

Departments of Dermatology (Drs Paltiel, Deng, and Gaspari), Rheumatology (Dr Mikdashi), and Neurology (Dr Alexeeva), University of Maryland, and Department of Allergy and Clinical Immunology, Johns Hopkins University (Drs Gober and Saini), Baltimore, Maryland.

CASE 1

A 41-year-old Asian woman with a history of HLA-B27–positive spondyloarthropathy was treated with adalimumab, 40 mg subcutaneously injected every other week, for arthritis involving both knees and the left ankle. The patient's arthritic symptoms improved considerably; however, after 5 injections she began to develop ISRs consisting of prominent edema, erythema, and pruritus at the injection sites on the abdomen and thighs. The initial reactions were localized to a small area within approximately 3 cm of injection site and appeared several hours after an injection, and subsequent ISRs developed more rapidly (within minutes) and involved an increasingly greater area. Edema and erythema around the injection site lasted 2 to 3 days after injection before gradual and complete resolution. After 2 months of therapy, the patient developed multiple pruritic erythematous scaly patches in a generalized distribution and discontinued the therapy. The dermatitis subsequently resolved with initiation of topical corticosteroids (clobetasol propionate, 0.05%, ointment applied to the body and hydrocortisone, 2.5%, cream applied to the face) and narrow-band UV-B therapy.

CASE 2

A 60-year-old white woman with a history of irritable bowel syndrome, psoriasis, and psoriatic arthritis, attended our dermatology clinic for treatment of persistent psoriasis and psoriatic arthritis. The patient was being treated with etanercept, 25 mg every week for 4 years, with incomplete clearance of psoriasis and persistent arthritic symptoms. The patient had been previously treated with methotrexate and phototherapy with limited success. Etanercept therapy was discontinued, and adalimumab therapy, 40 mg weekly for the first 4 weeks, was initiated. The patient initially experienced notable improvement of her arthritic symptoms and psoriasis but developed edema and erythema around the injection site within several hours after the second injection. Gradually, each subsequent injection of adalimumab resulted in a more prominent edema and erythema around the injection site and developed more rapidly. Each ISR lasted 2 to 3 days before gradual resolution. The patient subsequently experienced decreased efficacy of the drug in the treatment of psoriasis and psoriatic arthritis and discontinued the therapy.

METHODS

PIN-PRICK TESTING

Pin-prick testing was used to assess the presence of immediate-type hypersensitivity reaction to adalimumab in both patients using isotonic sodium chloride solution (normal saline), adalimumab vehicle (4.93 mg of sodium chloride, 0.69 mg of monobasic sodium phosphate dihydrate, 1.22 mg of dibasic sodium phosphate dihydrate, 0.24 mg of sodium citrate, 1.04 mg of citric acid monohydrate, 9.6 mg of mannitol, 0.8 mg of polysorbate 80, and water), and histamine (10 mg/mL in sterile normal saline) as controls. A drop of each solution was placed on pa-

tients' forearm, and skin was lightly pricked with a 27-gauge needle. A reading was performed 15 minutes after the pin prick. In addition, 0.1 mL (40 mg/0.8 mL) of adalimumab, 0.1 mL of vehicle, and 0.1 mL of normal saline were injected intradermally in the attempt to reproduce ISRs in both patients. Similar testing was performed on 1 patient (patient 3), who had no history of ISR to adalimumab after 9 months of therapy.

BASOPHIL ISOLATION
AND HISTAMINE RELEASE

Venous blood samples for basophil studies were collected into syringes containing 5mM EDTA from 2 patients with a history of ISRs to adalimumab, 1 patient (patient 3) with no history of ISRs to adalimumab, and 1 healthy, nonatopic control patient with no history of exposure to adalimumab. Mixed blood leukocytes were isolated by dextran sedimentation and then stimulated for histamine release with adalimumab (0.5–50 000 ng/mL) (Humira; Abbott Laboratories, Abbott Park, Illinois), human γ -globulin (50–50 000 μ g/mL) (Sigma-Aldrich, St Louis, Missouri), polyclonal goat antihuman IgE (0.1 μ g/mL), and N-formyl-methionine-leucine-phenylalanine (1 μ M) at 37°C for 45 minutes. Assays were performed in duplicate in standard buffers containing calcium as previously described.¹³ Histamine was quantified from supernatants using an automated fluorometric assay. Results are presented as the percentage of total histamine content minus the subject's spontaneous histamine release.

BASOPHIL SENSITIZATION

In a separate experiment, basophils from the same nonatopic, healthy control patient were isolated by dextran sedimentation. Basophil surface IgE was stripped by incubating the cells with lactic acid (pH 3.9) in normal saline for 3.5 minutes at room temperature.^{14,15} Following lactic acid stripping, serum from patient 1 was incubated with cells from the nonatopic donor at 37°C for 60 minutes in buffers containing heparin and EDTA. Then the cells were stimulated for histamine release to adalimumab (500–50 000 ng/mL) as described in the previous subsection. As a control to confirm removal of surface-bound IgE, cells were stimulated for histamine release by polyclonal goat antihuman IgE (0.1 μ g/mL) and showed a 59% decrease in response after stripping (14%) vs before stripping (34%), with subsequent recovery of response (42%) following incubation with serum from patient 1.

STATISTICAL ANALYSES

Quantitative data were analyzed for statistically significant differences between control and treatment groups using the GraphPad Instat Software Program (GraphPad Software, San Diego, California). Because multiple comparisons were examined, a 1-way analysis of variance was applied to the quantitative data. $P < .05$ was considered statistically significant.

RESULTS

Pin-prick testing resulted in the development of wheal and flare within 15 minutes at the adalimumab site in both patients with a history of ISRs to adalimumab (**Figure 1** and **Figure 2**). A similar reaction was seen with histamine (positive control), and no reactions were seen with adalimumab vehicle or normal saline. No reaction was observed with adalimumab in patient 3 with no history of ISRs to the drug. Intradermal injection of adalimumab in patients 1 and 2 reproduced signs and

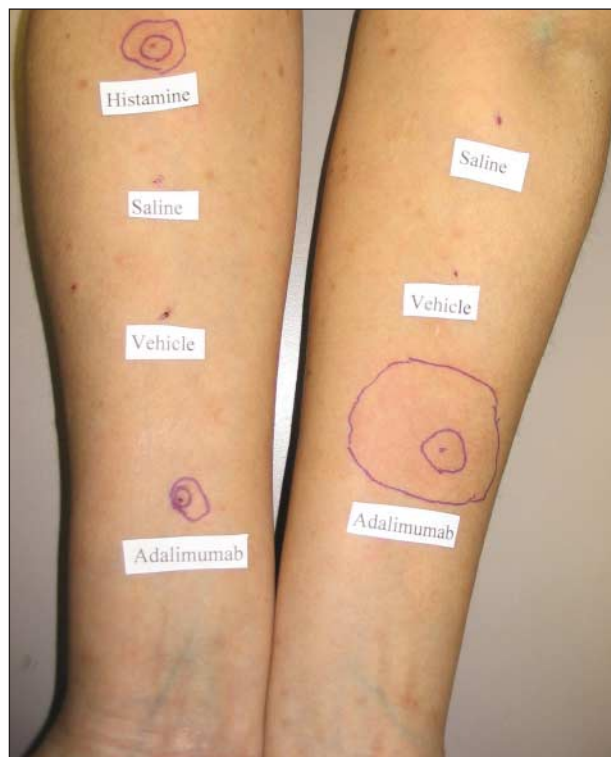


Figure 1. Skin reaction observed in patient 1 after pin-prick testing (right forearm) with adalimumab (40 mg/0.8 mL), using adalimumab vehicle, isotonic sodium chloride solution (normal saline), and histamine as controls. Injection site reaction (left forearm) was reproduced with an intradermal injection of 0.1 mL of adalimumab (40 mg/0.8 mL) but not with 0.1 mL of normal saline or 0.1 mL of adalimumab vehicle. (Inner demarcations outline areas of clinically evident induration, while outer demarcations outline areas of erythema.)

symptoms of ISRs in both patients, resulting in erythema and edema at the injection site (Figure 1 and Figure 2B). No notable reaction was seen at the injection sites of normal saline or vehicle control solutions. A skin biopsy at the adalimumab injection site 24 hours after injection in patient 1 revealed dermal edema with perivascular lymphocytic and eosinophilic infiltrates (Figure 3), which was consistent with an urticarial tissue reaction.

When peripheral blood leukocytes from both patients with history of ISRs and positive results of pin-prick testing with adalimumab were stimulated by adalimumab, significant *in vitro* histamine release was observed when compared with patient 3 ($P < .001$ for the 5000- and 50 000-ng/mL doses of adalimumab). No significant histamine release was observed when blood leukocytes from a healthy nonatopic adult volunteer with no history of exposure to adalimumab were stimulated by identical concentrations of adalimumab (Figure 4A). For a negative control, peripheral blood leukocytes from 3 patients and the control were stimulated with human γ -globulin at similar concentrations as used for adalimumab (data not shown), and none showed significant histamine release. As positive controls, peripheral blood leukocytes from each donor were also incubated with polyclonal goat antihuman IgE and *N*-formyl-methionine-leucine-phenylalanine and demonstrated *in vitro* histamine release to each stimulus (data not shown).

In the passive transfer experiment, basophils from the nonatopic, healthy donor were stripped with lactic acid

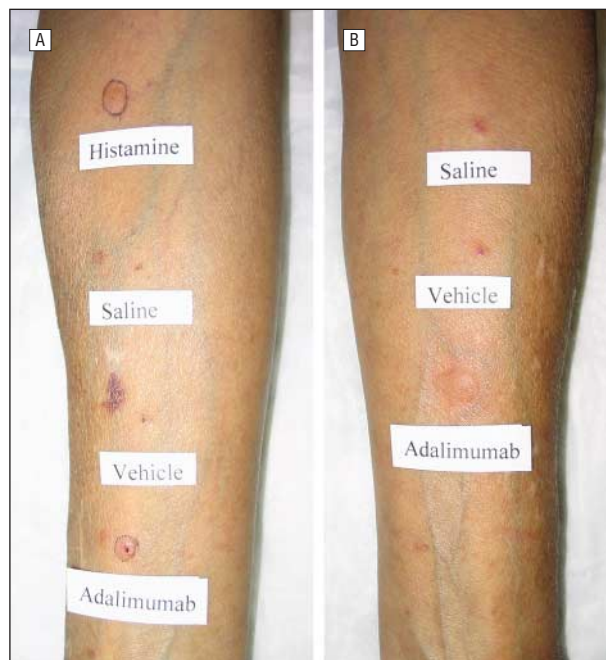


Figure 2. A, Reaction to pin-prick testing with adalimumab in patient 2 (40 mg/0.8 mL), using adalimumab vehicle, isotonic sodium chloride solution (normal saline), and histamine as controls; B, injection site reaction was observed with intradermal injection of 0.1 mL of adalimumab (40 mg/0.8 mL), 0.1 mL of normal saline, and 0.1 mL of adalimumab vehicle.

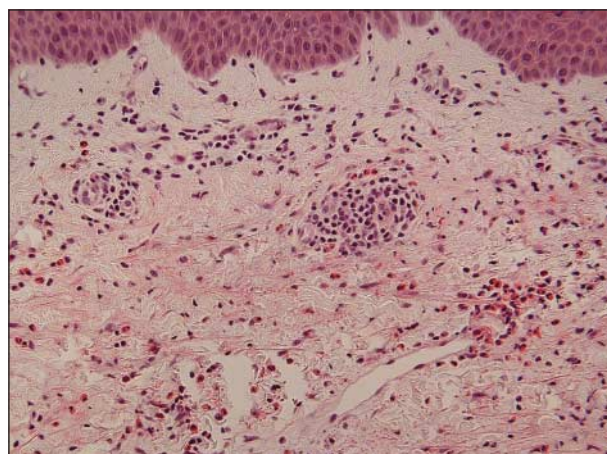


Figure 3. Histopathologic features of a urticarial tissue reaction seen in a patient after intradermal testing with adalimumab showing intradermal edema along with perivascular lymphocytic and eosinophilic infiltrate (hematoxylin-eosin, original magnification $\times 200$).

to remove donor surface-bound IgE and sensitized with serum from patient 1. On stimulation with adalimumab, sensitized basophils released considerable amounts of histamine (Figure 4B), supporting the presence of adalimumab-specific IgE in the serum of patient 1. The same nonatopic donor was used as a control for both the basophil histamine release and basophil sensitization experiments.

COMMENT

The advent of anti-TNF agents in recent years has provided an important armamentarium in the treatment of

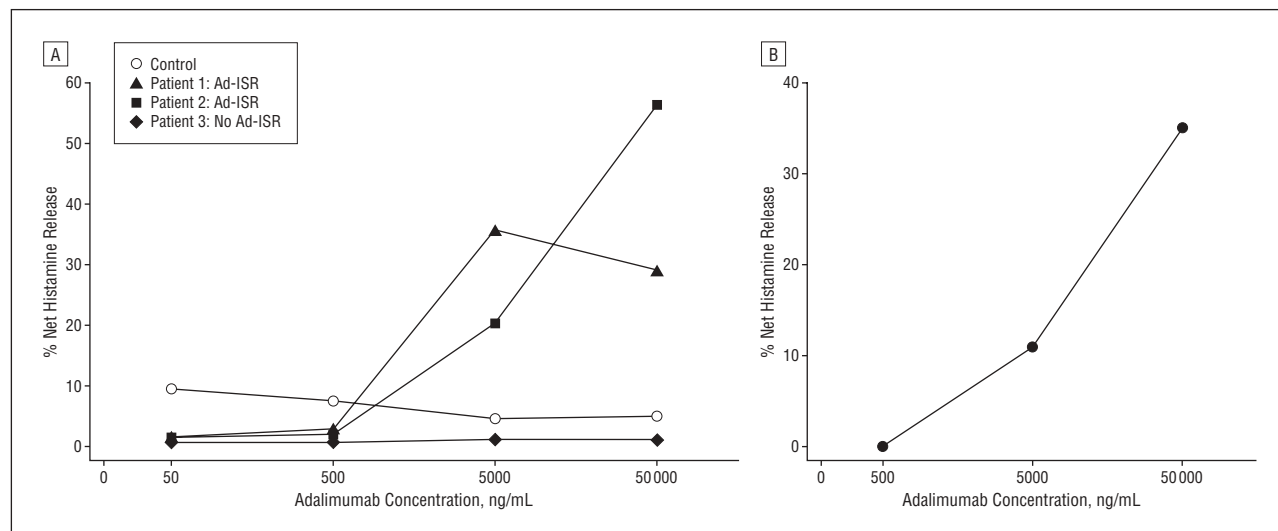


Figure 4. Basophil histamine release to adalimumab and passive basophil sensitization. A, Basophil histamine release from patients 1, 2, and 3 and a healthy nonatopic volunteer (control) following stimulation with adalimumab ($P < .001$, analysis of variance for the 5000- and 50 000-ng/mL doses of adalimumab). Ad-ISR indicates adalimumab-induced injection site reaction. B, Basophil histamine release from basophils from the healthy nonatopic control shown in panel A following sensitization with serum from patient 1 and then with adalimumab. This passive transfer experiment supports the presence of adalimumab-specific IgE antibodies in the serum of patient 1.

various dermatologic, rheumatologic, and gastrointestinal conditions. Although generally well tolerated, increasing use of these medications in clinical practice has led to the recognition of a variety of dermatologic adverse effects. Considering the increasing use of anti-TNF agents in dermatologic and other inflammatory conditions, it is important to augment the body of knowledge about adverse reaction and to elucidate their dermatopathologic and immunologic features.

Injection site reactions are common with subcutaneously administered anti-TNF agents and occur at a frequency of approximately 37% with etanercept¹⁶ and 20% with adalimumab.¹⁷ Zeltser et al¹² analyzed ISRs to etanercept and determined that the majority of the dermal infiltrate in these ISRS is composed of CD8⁺ T cells, indicating a cell-mediated T_H1 reaction suggestive of a lymphocyte-mediated delayed-type hypersensitivity reaction. We present the first report, to our knowledge, demonstrating the role of immediate-type I hypersensitivity reaction in the development of ISRs in 2 patients receiving adalimumab.

Type I hypersensitivity reaction is a manifestation of acute allergic reaction resulting from the release of preformed chemokines, granule-associated mediators, membrane-derived lipids, and cytokines and when an allergen interacts with IgE that is bound to mast cells or basophils by the α -chain of the high-affinity IgE receptor (Fc ϵ RI- α). The complex of allergen, IgE, and Fc ϵ RI on the surface of the mast cell triggers a noncytotoxic, energy-dependent release of histamine and tryptase and the membrane-derived lipid mediators leukotrienes, prostaglandins, and platelet-activating factor.¹⁸ These mast-cell mediators have a critical role in urticarial-type reactions that has been observed in 2 of our patients with ISRs to adalimumab.

Patient 1 also developed multiple pruritic erythematous patches in a generalized distribution within 2 months of starting adalimumab therapy. Similar eruptions have been reported in other patients receiving anti-TNF therapy¹⁹⁻²¹; however, the pathophysiologic mecha-

nisms of these eruptions remains to be elucidated.²² Patient 2 experienced decreased efficacy of adalimumab with continued use, and it is unclear if the adalimumab-specific IgE antibodies play a role in neutralizing the therapeutic effect of the drug.

In clinical trials with adalimumab, approximately 1% of patients experienced allergic reactions such as allergic cutaneous eruptions, anaphylactoid reaction, fixed drug reaction, nonspecified drug reaction, and urticaria.¹⁷ In addition, anaphylaxis and angioneurotic edema have been reported rarely in postmarketing experience with adalimumab.¹⁷ Our data suggest that some worsening ISRs to adalimumab are also allergic in nature and that further studies with a larger series of patients are warranted to determine whether type I hypersensitivity response is seen universally in patients with worsening ISRs to adalimumab.

Accepted for Publication: December 28, 2007.

Correspondence: Anthony A. Gaspari, MD, Department of Dermatology, University of Maryland, 405 W Redwood St, Sixth Floor, Baltimore, MD 21201 (agasp001@umaryland.edu).

Author Contributions: Drs Paltiel and Gaspari had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. *Study concept and design:* Gaspari. *Acquisition of data:* Paltiel, Gober, Deng, Alexeeva, Saini, and Gaspari. *Analysis and interpretation of data:* Paltiel, Gober, Deng, Mikdashi, Saini, and Gaspari. *Drafting of the manuscript:* Paltiel, Deng, Mikdashi, Alexeeva, Saini, and Gaspari. *Critical revision of the manuscript for important intellectual content:* Paltiel, Gober, Saini, and Gaspari. *Statistical analysis:* Gaspari. *Administrative, technical, and material support:* Paltiel, Gober, Deng, Mikdashi, Alexeeva, and Saini. *Study supervision:* Gaspari.

Financial Disclosure: Dr Gaspari has served as a consultant for Merck, Coria Labs, Amgen, and 3M; has re-

ceived honoraria from Astellas, Centocor, Graceway, Merck, Amgen, Abbott Laboratories, and Novartis; and has received grants from 3M and owns patents with 3M. Dr Saini has served as a consultant for Genentech and Novartis and has received grants from Genentech.

Funding/Support: Dr Gaspari has received funding from the National Institutes of Health, VA Merit Award, and National Institute of Arthritis and Musculoskeletal and Skin Diseases

Additional Contributions: Rita Fischelevich prepared materials used in the study.

REFERENCES

1. Mease PJ, Goffe BS, Metz J, VanderStoep A, Finck B, Burge DJ. Etanercept in the treatment of psoriatic arthritis and psoriasis: a randomised trial. *Lancet*. 2000; 356(9227):385-390.
2. Lipsky PE, van der Heijde DM, St Clair EW, et al; Anti-Tumor Necrosis Factor Trial in Rheumatoid Arthritis with Concomitant Therapy Study Group. Infliximab and methotrexate in the treatment of rheumatoid arthritis. *N Engl J Med*. 2000; 343(22):1594-1602.
3. Moreland LW, Schiff MH, Baumgartner SW, et al. Etanercept therapy in rheumatoid arthritis: a randomized, controlled trial. *Ann Intern Med*. 1999;130(6): 478-486.
4. Weinblatt ME, Keystone EC, Furst DE, et al. Adalimumab, a fully human anti-tumor necrosis factor alpha monoclonal antibody, for the treatment of rheumatoid arthritis in patients taking concomitant methotrexate: the ARMADA trial. *Arthritis Rheum*. 2003;48(1):35-45.
5. Lovell DJ, Giannini EH, Reiff A, et al. Long-term efficacy and safety of etanercept in children with polyarticular-course juvenile rheumatoid arthritis: interim results from an ongoing multicenter, open-label, extended-treatment trial. *Arthritis Rheum*. 2003;48(1):218-226.
6. Brandt J, Khariouzov A, Listing J, et al. Six-month results of a double-blind, placebo-controlled trial of etanercept treatment in patients with active ankylosing spondylitis. *Arthritis Rheum*. 2003;48(6):1667-1675.
7. Braun J, Brandt J, Listing J, et al. Treatment of active ankylosing spondylitis with infliximab: a randomised controlled multicentre trial. *Lancet*. 2002;359(9313): 1187-1193.
8. Lawson MM, Thomas AG, Akobeng AK. Tumour necrosis factor alpha blocking agents for induction of remission in ulcerative colitis. *Cochrane Database Syst Rev*. 2006;3:CD005112.
9. Hanauer SB, Snadborn WJ, Rutgeerts P, et al. Human anti-tumor necrosis factor monoclonal antibody (adalimumab) in Crohn's disease: the CLASSIC-I trial. *Gastroenterology*. 2006;130(2):323-333.
10. Keystone EC, Kavanaugh AF, Sharp JT, et al. Radiographic, clinical, and functional outcomes of treatment with adalimumab (a human anti-tumor necrosis factor monoclonal antibody) in patients with active rheumatoid arthritis receiving concomitant methotrexate therapy: a randomized, placebo-controlled, 52-week trial. *Arthritis Rheum*. 2004;50(5):1400-1411.
11. Maini R, St Clair EW, Breedveld F, et al; ATTRACT Study Group. Infliximab (chimeric anti-tumour necrosis factor alpha monoclonal antibody) versus placebo in rheumatoid arthritis patients receiving concomitant methotrexate: a randomised phase III trial. *Lancet*. 1999;354(9194):1932-1939.
12. Zeltser R, Valle L, Tanck C, Molyst MM, Ritchlin C, Gaspari AA. Clinical, histological, and immunophenotypic characteristics of injection site reactions associated with etanercept: a recombinant tumor necrosis factor [alpha] receptor: Fc fusion protein. *Arch Dermatol*. 2001;137(7):893-899.
13. Vasagar K, Vonakis BM, Gober LM, Viksman A, Gibbons SP Jr, Saini SS. Evidence of in vivo basophil activation in chronic idiopathic urticaria. *Clin Exp Allergy*. 2006;36(6):770-776.
14. MacGlashan DW, Bochner BS, Adelman DC, et al. Down-regulation of Fc (epsilon)RI expression on human basophils during in vivo treatment of atopic patients with anti-IgE antibody. *J Immunol*. 1997;158(3):1438-1445.
15. Saini SS, Klion AD, Holland SM, Hamilton RG, Bochner BS, MacGlashan DW Jr. The relationship between serum IgE and surface levels of FcεR on human leukocytes in various diseases: correlation of expression with FcεpsilonRI on basophils but not on monocytes or eosinophils. *J Allergy Clin Immunol*. 2000;106(3):514-520.
16. Enbrel (etanercept) [package insert]. Thousand Oaks, CA: Amgen; 2006.
17. Humira (adalimumab) [package insert]. North Chicago, IL: Abbott Laboratories; 2002.
18. Kay AB. Allergy and allergic diseases. *N Engl J Med*. 2001;344(1):30-37.
19. Flendrie M, Vissers WH, Creemers MC, de Jong EM, van de Kerkhof PC, van Riel PL. Dermatological conditions during TNF-alpha-blocking therapy in patients with rheumatoid arthritis: a prospective study. *Arthritis Res Ther*. 2005;7(3):R666-R676.
20. Cohen JD, Bournerias I, Buffard V, et al. Psoriasis induced by tumor necrosis factor-alpha antagonist therapy: a case series. *J Rheumatol*. 2007;34(2):380-385.
21. Sfrikakis PP, Iliopoulos A, Elezoglou A, Kittas C, Stratigos A. Psoriasis induced by anti-tumor necrosis factor therapy: a paradoxical adverse reaction. *Arthritis Rheum*. 2005;52(8):2513-2518.
22. Ritchlin C, Tausk F. A medical conundrum: onset of psoriasis in patients receiving anti-tumour necrosis factor agents. *Ann Rheum Dis*. 2006;65(12):1541-1544.

Archives Web Quiz Winner

Congratulations to the winner of our June quiz, Pan-kaj Salphale, MD, Department of Dermatology, Christian Medical College, Vellore, India. The correct answer to our June challenge was *generalized basoid follicular hamartoma syndrome*. For a complete discussion of this case, see the Off-Center Fold section in the July Archives (Fulchiero GJ Jr, Jones CR, Billingsley EM. Multiple facial papules and palmar pits. *Arch Dermatol*. 2008; 144[7]:933-938).

Be sure to visit the Archives of Dermatology Web site (<http://www.archdermatol.com>) to try your hand at the interactive quiz. We invite visitors to make a diagnosis based on selected information from a case report or other feature scheduled to be published in the following month's print edition of the Archives. The first visitor to e-mail our Web editors with the correct answer will be recognized in the print journal and on our Web site and will also receive a free copy of *The Art of JAMA II*.