

# EXPERT OPINION

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## Etanercept-induced injection site reactions: potential pathomechanisms and clinical assessment

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**Introduction:** Etanercept (ETN) is a tumor necrosis factor alpha (TNF- $\alpha$ ) antagonist used for the treatment of chronic inflammatory disorders. Injection site reactions (ISRs) are reported to be the most common adverse event of ETN therapy. While their mechanisms are not completely understood, the occurrence of ETN-ISRs could indicate a risk of systemic immune-mediated severe adverse drug reactions.

**Areas covered:** Based on two cases and a review of the literature, the characteristics and frequency of ETN-ISRs were assessed. This article discusses their potential mechanisms and clinical relevance, and provides recommendations for the management of patients presenting with ETN-ISRs.

**Expert opinion:** Basically, irritative and immune-mediated ISRs may be distinguished. The formation of anti-drug antibodies (ADAs) may promote immune-mediated ISRs that likely represent either anaphylactic type I reactions, or cutaneous Arthus-like type III reactions according to the Coombs and Gell classification. A differentiation between these reactions by clinical course and etanercept-skin testing may help to decide if ETN treatment should be stopped to avoid the development of more severe adverse drug reactions if ISRs occur.

**Keywords:** anti-drug antibodies, etanercept, immunogenicity, injection site reaction

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### 1. Introduction

Etanercept (ETN, Enbrel<sup>®</sup>) is a recombinant TNF-receptor IgG-Fc-fusion protein composed of the p75 TNF receptor genetically fused to the Fc domain of IgG1. Etanercept neutralizes the proinflammatory cytokine tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and lymphotoxin- $\alpha$  [1]. It is an effective and approved treatment option for several immune-mediated inflammatory diseases including chronic plaque psoriasis, psoriatic arthritis (PsA), rheumatoid arthritis (RA) and ankylosing spondylitis (AS). Efficacy and safety of ETN have been well documented [2-4]. Injection site reactions (ISRs) are reported to be the most common adverse event of ETN therapy. The frequency of ISRs has been recorded in various clinical trials (Table 1a, 1b) [5-15]. Although ETN is widely used as disease-modifying anti-rheumatic drug, the mechanisms of ETN-induced ISRs are not yet completely understood, may be diverse, and the clinical relevance has not been systematically assessed [2,16-18]. Based on two cases of immune-mediated ETN-induced ISRs (ETN-ISRs) and an extensive literature review, we discuss the potential mechanisms and try to define criteria, which may help to decide if a particular ISR may indicate a risk for injection-associated adverse events upon further ETN exposure and require cessation of treatment.

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**Figure 1.** A 56-year-old woman with chronic plaque psoriasis (case #1) presented with erythematous and edematous plaques that sequentially appeared on her abdomen at the last three injection sites.

## 2. Patients and methods

### 2.1 Patients

Patients were treated at the Psoriasis Centre of the Department of Dermatology of the Ludwig-Maximilian-University of Munich with ETN according to the approved indication for moderate-to-severe chronic-plaque psoriasis.

### 2.2 Skin testing

Prick testing was performed using commercially available ETN (Enbrel®). The commercially available standard concentration (50 mg/ml) was diluted 1:10 with 0.9% NaCl (final concentration 5 mg/ml). Intradermal injection was performed with 100 µl of ETN (Enbrel®, 50 mg/ml) diluted 1:10 with 0.9% NaCl (final concentration 5 mg/ml).

### 2.3 Ouchterlony double-diffusion assay

Flat-bed agar plates (1.3% agar, 0.9% NaCl, thickness 4 mm) with a central well (11 mm in diameter) and six surrounding wells (6 mm in diameter) were used. The central well was loaded with 100 µl patient serum and the outer wells with 35 µl of ETN (Enbrel®) in series dilutions with concentrations ranging from 1 mg/ml to 0.975 µg/ml. The plates were incubated for 48 h at room temperature. The patients' blood samples had been taken 7 days after discontinuation of ETN therapy. Sera from four healthy individuals never exposed to ETN served as controls.

### 2.4 Literature review

For literature review, we searched PubMed database [19] from March 1999 to March 2012 for journal articles reporting ISRs during treatment with ETN, using different combinations of the following search terms: ETN, psoriasis, rheumatoid arthritis, ankylosing spondylitis, randomized, controlled, clinical trial, safety, adverse events, injection site reaction, immunogenicity, anti-ETN antibodies. We analyzed the frequency and characteristics of ETN-induced injection site

reactions (ETN-ISRs) reported in randomized controlled trials (RCTs) and case reports. We also reviewed the frequency of the anti-ETN antibodies and methods for their detection in RCTs.

## 3. Results

### 3.1 Cases

**Case #1.** A 56-year-old woman with a 50-year history of chronic plaque psoriasis started therapy with ETN (50 mg s.c., twice weekly). She applied the first four injections at the abdominal skin. Approximately 20–24 h after the fourth injection, she developed an itching erythematous and edematous plaque of 6 cm in diameter at the injection site. Another 12 h later similar lesions occurred sequentially also at the sites of the third, second and finally after 48 h of the first injection (Figure 1). Prick testing with ETN was negative. An intradermal ETN injection induced a persistent inflammatory lesion with a peak intensity after 24 h that resembled those on the abdomen. A biopsy taken from the lesion at the test site revealed a perivascular inflammatory infiltrate composed mostly of neutrophilic granulocytes without eosinophils (Figure 2). Because of the intensity of the ISR, ETN treatment was terminated. The abdominal lesions were treated with topical glucocorticosteroids and resolved within 2 weeks.

**Case #2.** A 60-year-old man with a 23-year history of chronic plaque psoriasis was retreated after four years with ETN (25 mg twice weekly). Within 1 h after the sixteenth retreatment injection he developed an urticaria-like lesion of 1.5 cm in diameter at the injection site that resolved after several hours. The patient continued therapy, however, clinically similar but more intense lesions appeared at the following injections. While the prick tests with ETN at two different concentrations remained negative, the intradermal injection induced a wheal within a few minutes, which persisted a few hours and indicated immediate-type hypersensitivity against ETN. Treatment was terminated in order to avoid an anaphylactic reaction.

In both patients prick testing with latex and standard allergens were negative. In Ouchterlony double-diffusion assay, sera from both patients formed whitish precipitations with ETN. In patient 1 precipitation lines were seen at ETN concentrations between 15.625 and 500 µg/ml (Figure 3A), in patient 2 at concentrations of 31.25, 15.625 and 7.8 µg/ml. No precipitation lines were observed with sera from healthy controls (Figure 3B).

### 3.2 Frequency of ISRs in randomized controlled trials

In RCTs conducted in adult patients with moderate-to-severe chronic plaque psoriasis, ISRs were reported in up to 18.3% of patients receiving ETN 50 mg twice weekly (BIW), in 16.7% of patients receiving ETN 50 mg once weekly (QW), in up to 17% of patients with ETN 25 mg BIW and in 11% of those on ETN 25 mg QW (Table 1a) [5–9]. Injection site reactions were noted in 33.8% of psoriasis patients treated

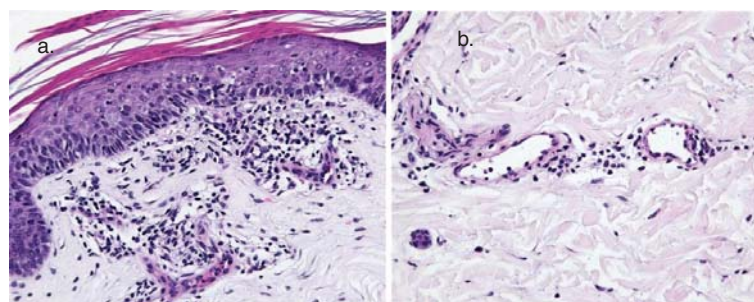


**Figure 2. (A, B)** A biopsy taken from the test site 24 h after intradermal injection of etanercept (5 mg/ml) revealed a mixed perivascular inflammatory infiltrate with neutrophilic granulocytes as characterized by polymorph segmented nuclei preferentially in and around blood vessels, without eosinophils (HE  $\times 40$ ).

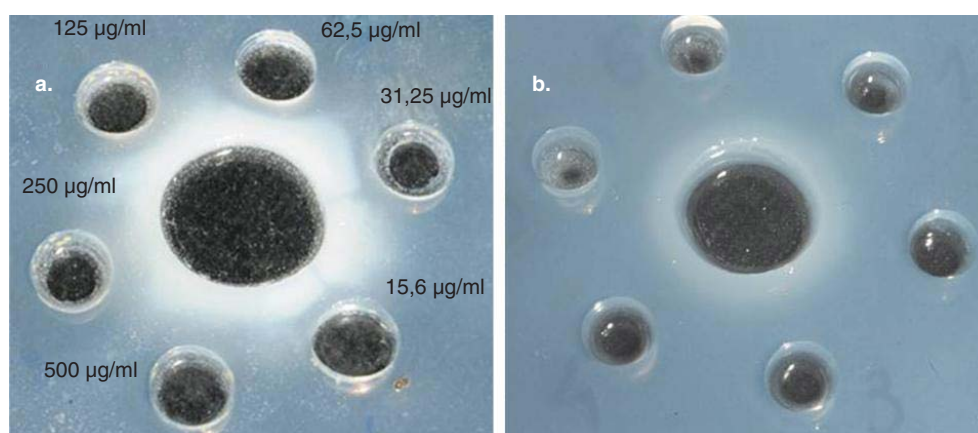


**Figure 3. Ouchterlony double-diffusion assay.** Serum from patient #1 formed precipitations lines with etanercept (A). No precipitation lines were observed with serum from a healthy control (B).

with ETN 0.8 mg/kg QW (Table 1a) during childhood and adolescence [10]. In RCTs for RA, ISRs were reported in up to 49% of patients receiving 25 mg ETN BIW and in 43% receiving 10 mg BIW (Table 1b) [11-14]. In patients with PsA receiving 25 mg ETN, QW ISRs were reported in 36% of subjects (Table 1a) [15]. The type of ISRs was usually not described in detail. Injection site reactions appeared mainly within the first three months of therapy, were usually mild to moderate and most often resolved with continuation of therapy. Only a few patients discontinued from the studies due to ISRs [5-15].

A retrospective analysis of 103 patients treated with ETN regardless of the underlying disease identified ISRs in 20% of cases [16]. They occurred mostly within the first two months of therapy one to two days after the last injection and the median number of injections at the time of onset was 4 (range 3 – 8). Forty per cent of the ISRs appeared also at previous injection sites after the last injection and were termed ‘recall’ ISRs. Patient with ISRs generally responded well to treatment and there was no necessity of therapy interruption. The

lesions resolved within 2 – 3 days. In all but one patient, ISRs disappeared with ongoing treatment (Table 2) [16].

### 3.3 Reports on ETN-specific antibody formation

Etanercept is a recombinant human (rhu) fusion protein composed of two extracellular domains of the p75 TNF receptor genetically fused to the Fc domain of IgG1 [1]. The fusion region, which links two primarily unrelated human amino acid sequences likely constitutes a neo-antigen capable of inducing fusion site-specific immune responses [20,21]. The incidence of ETN-specific anti-drug antibodies (ADAs) has been analyzed in various studies by enzyme-linked immunosorbent assay (ELISA) or radioimmunoassay (RIA). Although rhu fusion proteins seem to be less immunogenic than chimeric mouse/human biologic agents, ETN antibodies were identified by ELISA in 0.6 – 18.3% of patients in RCTs (Table 3). They were determined as non-neutralizing, as they did not influence treatment efficacy. Furthermore, they did not have an apparent effect on the safety profile although as pointed out by some authors the small patient

**Table 1a. Frequency of ISRs during ETN therapy in patients with chronic plaque psoriasis and psoriatic arthritis in RTCs.**

| Ref. | Type of trial | Disease | No of patients | Etanercept dosage (mg) | Frequency of ISR (%) |
|------|---------------|---------|----------------|------------------------|----------------------|
| [5]  | RCT           | CPPs    | 652            | Pbo                    | 7                    |
|      |               |         |                | 25 QW                  | 11                   |
|      |               |         |                | 25 BIW                 | 17                   |
|      |               |         |                | 50 BIW                 | 13                   |
| [6]  | RCT           | CPPs    | 112            | Pbo                    | 0                    |
|      |               |         |                | 25 BIW                 | 9                    |
| [7]  | RCT           | CPPs    | 583            | Pbo                    | 6                    |
|      |               |         |                | 25 BIW                 | 13                   |
|      |               |         |                | 50 BIW                 | 18                   |
| [8]  | RCT           | CPPs    | 618            | Pbo                    | 6.1                  |
|      |               |         |                | 50 BIW                 | 12.2                 |
| [9]  | RCT           | CPPs    | 142            | Pbo                    | 2.2                  |
|      |               |         |                | 50 QW                  | 16.7                 |
| [10] | RCT           | CPPs*   | 211            | Pbo                    | 4.70                 |
|      |               |         |                | 0.8/kg QW              | 33.80                |
| [15] | RCT           | PsA     | 205            | Pbo                    | 9                    |
|      |               |         |                | 25 BIW                 | 36                   |

\*In children and adolescents.

BIW: Twice weekly; CPPs: Chronic plaque psoriasis; No: Number; Pbo: Placebo; PsA: Psoriasis arthritis; QW: Once weekly; RCT: Randomized controlled trial.

**Table 1b. Frequency of ISRs during ETN therapy in patients with rheumatoid arthritis in RTCs.**

| Ref. | Type of trial | No of patients | Etanercept/MTX dosage (mg) | Frequency of ISR (%) |
|------|---------------|----------------|----------------------------|----------------------|
| [11] | RCT           | 89             | Pbo+ MTX 10 – 25 QW        | 7                    |
|      |               |                | ETN 25 BIW+ MTX 10 – 25 QW | 42                   |
| [12] | RCT           | 234            | Pbo                        | 13                   |
|      |               |                | ETN 10 BIW                 | 43                   |
|      |               |                | ETN 25 BIW                 | 49                   |
| [13] | RCT           | 632            | MTX 19*QW                  | 7                    |
|      |               |                | ETN 10 BIW                 | 30                   |
|      |               |                | ETN 25 BIW                 | 37                   |
| [14] | RCT           | 686            | ETN 25 BIW                 | 2                    |
|      |               |                | MTX up to 20 QW            | 2                    |

\*Mean value.

BIW: Twice weekly; ETN: Etanercept; Mtx: Metotrexate; No: Number; Pbo: Placebo; QW: Once weekly; RCT: Randomized controlled trial.

numbers with proven ETN antibodies provided only low statistical power [5,7,8,11-13,15,22-25].

### 3.4 Case reports of ETN-ISRs in the literature

Several reports have provided a more detailed analysis of ETN-ISRs (Table 2) [16,18,26-32]. Zeltser *et al.* [16] performed histological analyses of biopsies taken from two primary and one recall ETN-ISRs at their peak intensity 24 h after ETN injection. They showed a mild-to-moderate perivascular inflammatory infiltrate consisting mostly of lymphocytes, moderate numbers of partly degranulated eosinophils, neutrophils and macrophages in all three cases. Immunophenotyping revealed a predominance of HLA-DR<sup>+</sup>/CD4<sup>+</sup>/CD8<sup>+</sup> T cells. The authors therefore postulated that ETN-ISRs may represent delayed type hypersensitivity reactions (DTH)

mediated by CD8<sup>+</sup> T lymphocytes, which may resolve with ongoing treatment due to the development of immune tolerance. Similarities to fixed drug eruptions were pointed out as well. Gonzales-Lopez *et al.* [26] reported two cases of recall ETN-ISRs in RA patients. Erythematous-edematous plaques with pruritus appeared at all previous injection sites within 24 h after the fourth (patient 1) or the third (patient 2) ETN injection. A histological and immunophenotypic analysis in both cases revealed an inflammatory infiltrate composed mostly of CD4<sup>+</sup> T lymphocytes. The authors proposed that ETN-ISRs had resulted from classical DTH and were mediated by CD4<sup>+</sup> T lymphocytes. Edwards *et al.* [27] reported a case of persistent and worsening recall ISRs that occurred in the tenth month of ETN treatment in an RA patient. Large (20 cm in diameter), well-demarcated erythematous and

Table 2. Review of the literature regarding immune-mediated etanercept-induced injection site reactions (ETN-ISR).

| Ref. | Type of publication    | Type of reaction                                 | Time course after injection     | Histopathologic analysis                                                                                             | Additional analyses                                                                                     | Interpretation                                                                         |
|------|------------------------|--------------------------------------------------|---------------------------------|----------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------|
| [16] | Retrospective analysis | Primary and recall (~40%) ISRs                   | 1–2 days p.i., max. within 24 h | Perivascular infiltrate: mostly lymphocytes; eosinophils, neutrophils and macrophages                                | IH: mostly HLA-DR <sup>+</sup> /CD4 <sup>+</sup> /CD8 <sup>+</sup> T cells                              | CD8 <sup>+</sup> T cell-mediated DTH, with induction of tolerance<br>Similarity to FDE |
| [26] | CR                     | Recall ISR                                       | 24 h p.i.                       | Superficial perivascular infiltrate: lymphocytes and eosinophils                                                     | IH: mostly CD4 <sup>+</sup> T-cells                                                                     | CD4 <sup>+</sup> T cell-mediated DTH                                                   |
| [18] | CR                     | Recall ISR                                       | Not given precisely             | Perivascular infiltrate: mostly lymphocytes                                                                          | IH: mostly CD4 <sup>+</sup> T-cells                                                                     | CD4 <sup>+</sup> T cell-mediated DTH                                                   |
|      |                        | Recall ISR                                       | Not given precisely             | Superficial, perivascular infiltrate: lymphocytes and eosinophils                                                    | DIF: weak staining of IgM and C3 at the dermo-epidermal junction                                        | Mechanism unclear                                                                      |
| [27] | CR                     | Recall ISR                                       | Within 24 h                     | Superficial and deep, perivascular infiltrate: lymphocytes, histiocytes, eosinophils, nuclear fragments, neutrophils | Not done                                                                                                | Urticarial vasculitis-like reaction                                                    |
| [29] | CR                     | Recall ISR                                       | Not given precisely             | Not done                                                                                                             | Not done                                                                                                | DTH                                                                                    |
| [28] | CR                     | Recall ISR                                       | Not given precisely             | Not done                                                                                                             | Not done                                                                                                | Similarity to FDE<br>Precise mechanism unclear                                         |
| [31] | CR                     | ISR at last injection                            | Onset 6 h p.i.                  | Not done                                                                                                             | Skin testing with ETN:<br>Intradermal testing: positive after 15 min<br>Prick testing: positive         | Immediate type reaction, possibly IgE-mediated                                         |
|      |                        | ISR at last injection                            | Onset 5 min p.i.                | Not done                                                                                                             | skin testing with ETN:<br>Intradermal testing: positive after 15 min;<br>Prick testing: positive        | Immediate-type reaction, possibly IgE-mediated                                         |
| [32] | CR                     | ISR at last injection plus generalized urticaria | Within 10–20 min p.i.           | Not done                                                                                                             | Skin testing with ETN:<br>Intradermal testing: positive at immediate reading<br>Prick testing: negative | Immediate type reaction, possibly IgE-mediated                                         |
| [30] | CR                     | ISR at last injection                            | Within 12–24 h                  | Superficial and deep perivascular and interstitial infiltrate: lymphocytes, numerous eosinophils                     | Intradermal testing with benzyl alcohol: negative                                                       | Wells syndrome (eosinophilic cellulitis)                                               |

CR: Case report; DIF: Direct immunofluorescence; DTH: Delayed type hypersensitivity reaction; FDE: Fixed drug eruption; h: Hours; IH: Immunohistochemical phenotyping; min: Minutes, p.i: Post injection; Recall ISR: ISR at the last and subsequently previous injection sites.

indurated plaques appeared within 24 h after ETN injection. Histological analysis revealed a superficial and deep perivascular infiltrate consisting mostly of lymphocytes, histiocytes, eosinophils as well as nuclear fragments indicative of leukocytoclasia and occasionally intact neutrophils. The authors classified those lesions as urticarial reaction with some features of urticaria vasculitis. During ongoing therapy, even with a decrease in the ETN dose the worsening of ISRs and severe recall ISRs were noted. Other reports showed that recall ETN-ISRs at the last and subsequently at all previous injection sites may appear already very early in the course of treatment from the second ETN injection on [28,29]. Murphy *et al.* [18] observed an ISR developing after the fourth ETN injection. The lesion was erythematous and indurated, 12 cm in diameter and showed a perivascular infiltrate with lymphocytes and eosinophils as well as weak staining of IgM and C3 at the dermo-epidermal junction. Therapy was continued and subsequent ISR were mild. Winfield *et al.* [30] reported the development of erythematous, slightly indurated pruritic lesions within 12 – 24 h after the first and the second injections of ETN in a female RA patient, previously treated with adalimumab. Lesions resolved after treatment with prednisone 40 mg/d and topical steroids. Because histological analysis revealed superficial and deep perivascular and interstitial infiltrates with lymphocytes and numerous eosinophils surrounding collagen fibers, the authors postulated an ETN-directed T<sub>H</sub>2-mediated eosinophilic cellulitis. Bennuci *et al.* [31] presented two cases of ETN-ISRs in RA patients. In the first one the ISRs appeared 6 h after the second ETN injection. In the second case the reaction occurred in the fifth year of ETN therapy, 5 min after injection with a peak intensity after 30 min and a duration of four days. In both cases prick tests were positive. Intradermal tests were positive at immediate reading and negative at the late reading. The authors suggested immediate type hypersensitivity and discussed similarities to IgE-mediated hymenoptera sting reactions (Table 2). Bavbek *et al.* [32] reported on a patient with AS who developed an ISR followed by whole-body urticaria within 10 – 15 min after the twenty-second ETN injection. Prick testing with ETN was negative whereas intradermal testing was positive at immediate reading. The subsequent injection of ETN diluted 1:10 resulted in urticaria without ISR that persisted for a month and required treatment with cetirizine 10 mg daily. The authors decided to subject the patient to a desensitization protocol. Etanercept therapy was continued with mild ISRs and redness limited to legs (Table 2).

#### 4. Discussion

The review of the literature reports on ETN-induced ISRs suggested two main pathomechanisms of their development: either irritative or immune-mediated. The majority of ETN-ISRs reported in clinical trials are interpreted as irritative reactions resulting from, for example, high concentrations

of ETN, improper injection techniques or vehicle components [17]. The true frequency of either reaction type is, however, hard to estimate because in RCTs no precise analyses have been performed [5-16]. Only one report proposed that ~ 40% of ISRs may be immune mediated [16]. Irritative reactions usually appear on the first application, resolve spontaneously over time and require neither discontinuation of treatment nor particular diagnostic procedures. Irritation, however, cannot account for recall ISRs, delayed ISRs or persistent and worsening ISRs [33]. These ETN-ISRs seem to reflect specific immune-mediated reactions against the drug. They require a first exposure, which usually is well tolerated but induces sensitization of T cells and the formation of ETN-specific antibodies. Injection site reactions may then develop from the second injection on according to the mounting of an ETN-directed immune response. Contrary to irritative ISRs the immune-mediated local reactions should then occur after all subsequent injections. However, this latter criterion is not absolutely strict since decreasing intensity of ISRs over time may also indicate the development of immune tolerance [33]. Information on the potential ISR mechanism largely refers to case reports.

The immunogenicity of ETN is not completely understood [21,34-36]. Histopathologic analysis showed the presence of T cells, but no clear reaction pattern can be concluded from the few cases since bystander recruitment of T cells may accompany any inflammatory reaction and the specific role of these infiltrating T cells remains to be defined [16,18,26-29]. Differences between the results might be explained by the duration of therapy, co-medication, depth and dose of injection or time point of biopsy. Furthermore, neutralization of TNF- $\alpha$  by ETN itself might modify the phenotype of an ETN-directed immune response.

A predominant role of antibodies in the ETN-ISRs appears more likely than DTH. Formation of ETN-specific antibodies was reported in up to 18.3% of the patients in RCTs (Table 3) [5,7,8,11-13,15,22-25]. In our two patients with immune-mediated ISRs, Ouchterlony double-diffusion assay analyzing serum reactivity against ETN showed whitish precipitations indicative of ETN-specific antibodies.

The binding epitopes on ETN as well as isotypes of ETN antibodies have not been clearly defined yet. More precise studies concerning another rhu fusion protein, lenercept (human p55 TNF receptor--IgG1 fusion protein) demonstrated an induction of non-neutralizing IgM and IgG ADAs in a substantial number of patients. These varied significantly in titers and kinetics of appearance among individuals and were mainly directed against linear epitopes in close proximity to the fusion site [36]. IgG or IgM anti-ETN antibodies may result in immune complex (IC) formation and complement activation with the development of IC-mediated diseases, for example, local Arthus reactions or systemic serum sickness, vasculitis or nephritis [33]. Cases of leukocytoclastic or necrotizing vasculitis during ETN therapy have been reported [37-39]. Formation of immediate type IgE or

**Table 3. Frequency of ETN antibodies in randomized clinic trials.**

| Ref. | Disease | Frequency of detection (%) | Methodology          | Effect on efficacy or safety profile |
|------|---------|----------------------------|----------------------|--------------------------------------|
| [5]  | CPPs    | 0.8                        | ELISA, binding assay | No                                   |
| [7]  | CPPs    | 1.6                        | ELISA, binding assay | No                                   |
| [8]  | CPPs    | 18.3                       | ELISA                | No                                   |
| [15] | CPPs    | 0                          | ELISA                | No                                   |
| [22] | CPPs    | 3                          | ELISA                | No                                   |
| [23] | RA      | 5.6                        | ELISA                | No                                   |
| [13] | RA      | < 3                        | ELISA                | No                                   |
| [12] | RA      | 0.6                        | ELISA                | No                                   |
| [24] | JIA     | 8                          | Not specified        | No                                   |
| [25] | AS      | 0                          | Radioimmunoassay     | No                                   |
| [11] | RA      | 0.6                        | ELISA                | No                                   |

AS: Ankylosing spondylitis; CPPs: Chronic plaque psoriasis; JIA: Juvenile idiopathic arthritis; RA: Rheumatoid arthritis.

**Table 4. Recommendations for the management of patients presenting etanercept-induced injection site reactions (ETN-ISR).**

|                                                 | Irritative ISRs            | Immediate type I ISRs                                                                                                    | Arthus-like type III ISRs                                                          |
|-------------------------------------------------|----------------------------|--------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------|
| Onset                                           | Already at first injection | After the second reaction or later                                                                                       | After the second reaction or later                                                 |
| Course                                          | Rapid decline              | Onset within the first 20 min after injection, disappearance within 2 – 3 h, possibly urticaria or anaphylactic reaction | Onset several hours after injection, persistence for several days                  |
| Recall phenomenon                               | No                         | No                                                                                                                       | Yes                                                                                |
| Etanercept skin testing (prick and intradermal) | Negative                   | Immediate reaction (within 20 min) showing an urticarial lesion with wheal formation                                     | Delayed reaction within 6 – 20 h showing an inflammatory response for several days |
| Treatment                                       | Continue                   | Stop or desensitization under intensive stand care                                                                       | Decide individually in each case                                                   |

IgG4 antibodies might predispose patient to local or systemic anaphylactic reactions.

The data on ETN-ISRs summarized here suggest that the immune-mediated ETN-ISRs may be primarily mediated by ETN-specific antibodies with a secondary bystander T-cell recruitment for three major reasons. **Reason 1:** In general, the normal physiological immune response to a foreign, soluble protein is immunoglobulin-mediated [33]. Several analyses have shown that ETN-ISRs occur in the presence of ETN-antibodies (Table 3). The induction of antibodies varies inversely with the induction of DTH [40]. **Reason 2:** The time course of the ISRs is not consistent with a DTH reaction. In humans DTH reactions appear 48 – 72 h after application of antigen. The immune-mediated ETN-ISRs and the skin tests with ETN, however, have a much more rapid onset (Table 2). Moreover, ISRs showed different time courses and clinical manifestations in various patients. Skin tests with ETN became positive within a few minutes or a few hours. In our first case, the ISRs peaked in intensity 20 – 24 h after the fourth injection at the last and subsequently at all previous injection sites. Skin testing produced an inflammatory reaction visible already after 6 – 8 h with a peak intensity after

18 – 24 h after injection. This case clinically resembled those reported by Zelster *et al.* [16], Gonzales-Lopez *et al.* [26], Papadavid *et al.* [28], Rajakulendran *et al.* [29] and Edwards *et al.* [27]. In all of them ISRs appeared also at previous injection sites simultaneously with or sequentially after the last injection. Time course, proof of ETN-specific antibodies in the Ouchterlony double diffusion assay and the histopathology with neutrophilic granulocytes in the perivascular inflammatory infiltrate are consistent with a local antibody-mediated inflammatory response induced by the formation of immune-complexes against injected antigen and subsequent complement activation. This type of pathologic immune-response by definition is an Arthus reaction [41]. Cutaneous Arthus reactions (type III hypersensitivity according to Coombs and Gell) are clinically characterized by erythematous, edematous and potentially hemorrhagic changes such as reported by Zelster *et al.* [16] with peak intensity after 8 – 24 h. They result from skin injection of protein antigens in the presence of circulating antigen-specific antibodies. This leads to the local deposition of ICs, activation of the complement cascade, recruitment of neutrophilic granulocytes and further development of an inflammatory

response [42-47]. Neutrophils were found prominently in the histopathologic analysis of the skin-test reaction of our case 1 and occasionally in the other case reports on ETN-ISR [16,27]. Activation of skin mast cells via Fc $\gamma$ RIII, which release TNF- $\alpha$  was shown to play a prominent role in Arthus reactions [43,44]. TNF- $\alpha$  promotes the recruitment of neutrophilic granulocytes that contribute to edema formation, tissue destruction and hemorrhagia [44,47]. Since ETN neutralizes the biologic activity of TNF- $\alpha$ , it may modulate the course and appearance of an evolving ETN-directed Arthus reaction, change the pattern of infiltrating cells, for example, from neutrophilic to eosinophilic granulocytes observed by others [16,18,26,27,30] and confuse clinical and histopathological interpretation. **Reason 3:** DTH is a manifestation of T-cell-mediated immunity. DTH is differentiated into tuberculin-type, Jones-Mote type and contact hypersensitivity [48]. Jones-Mote type is considered as a delayed cutaneous reaction in humans and animals sensitized by skin injection of heterologous proteins [49]. Jones-Mote-type DTH reactions are characterized by a strong infiltration with basophilic granulocytes [50]. This cell type has not been observed to predominate in any of the histopathologic analyses of ETN-ISRs. Thus, a primary role of DTH in ETN-ISRs according to the current immunological DTH-classification appears unlikely. Instead, the T cells infiltrating ETN-ISRs are likely recruited secondary due to the activation of the complement during the immune-complex formation, which may provide essential signals for recruitment of CD4<sup>+</sup> and CD8<sup>+</sup> T cells [51]. These T cells then may act as regulators of the humoral immune response [33] and be responsible for the tolerance phenomenon seen in many patients with immune-mediated ISRs.

In our second case, the ISR appeared within 1 h after the sixteenth retreatment injection without a recall phenomenon in previous injection sites. Intradermal testing became positive within a few minutes, showed an urticaria-like phenotype and resolved spontaneously after several hours. This time course resembles cases reported by Benucci *et al.* [31] and Baybek *et al.* [32]. In all these cases, prick or intradermal testing with ETN produced immediate-type reactions within 15 – 20 min. This corresponds to an immediate-type hypersensitivity, which is mediated by antibodies of IgE- or IgG4-isotypes and represents anaphylactic type I hypersensitivity reactions of Coombs and Gell.

Taken together, the immune-mediated ISRs seem to be diverse (Table 2). It is likely that ETN-specific antibodies are pathogenetically involved and that their particular immunoglobulin isotype defines the particular type of ISR. Immune-mediated ISRs should be considered if the reactions appear with delayed onset after initiation of therapy, that is, from the second injection on, show recall phenomena or manifest as persistent and worsening reactions. According to the kinetics they should be classified into immediate (type I) and IC-mediated (type III) hypersensitivity that appear 20 min (type I) or within 8 – 12 h (type III) after ETN injection. This differentiation may be clinically important as

several cases of urticaria have been reported during ETN therapy [46,52] and the ETN product information lists anaphylactic reactions as a potential severe adverse event. Usually no specific routine *in vitro* test is available to define the presence and isotype of ETN-specific antibodies. Thus, a decision for maintaining or stopping treatment may require a more precise clinical assessment. It should assess the respective ISR history and involve ETN skin testing. Skin testing should be started with a prick test using the commercially available ETN preparations; however, its results may be inconclusive as the size of the molecule may hamper a rapid penetration to the dermal mast cells, which are the actual histamine-releasing cells. Therefore, in cases of negative prick test results an intradermal injection should be performed, starting with a 1:100 dilution of the commercial ETN preparation. Read out should be done immediately, after 20 – 30 min and after 6 and 24 h. If immediate reading shows a wheal-like reaction, IgE- or IgG4-mediated immediate type-I hypersensitivity should be considered and ETN treatment should be stopped to avoid the risk of systemic anaphylactic reactions upon further ETN exposure. The decision for desensitization may depend on the actual availability of alternative TNF blockers. According to the clinical experience, somewhat delayed immune-mediated ISRs occurring after 6 – 8 h and peaking 24 h or later after injection are usually not hazardous and may decline with prolonged ETN application, indicating the development of some type of immune tolerance. Therefore, if tolerated by a patient, ETN treatment may be continued in these cases, because the formation of antibodies is not associated with a loss of efficacy. The patient should, however, be informed about the risk and symptoms of serum sickness with necrotizing vasculitis that rarely was observed during ETN exposure [38,39]. These recommendations might help to increase the safety of treatment, when immune-mediated ISRs are suspected (Table 4).

## 5. Conclusions

ETN-ISRs occur frequently upon s.c. injection irrespective of the indication. They are either irritative or immune-mediated likely involving the formation of ETN-specific antibodies. Usually, ETN-ISRs are harmless, decrease over time, are not associated with a loss of efficacy and do not require cessation of ETN treatment. In select cases skin testing may help to distinguish type I IgE-mediated hypersensitivity reactions to identify the risk of systemic anaphylaxis. Each case of ETN-ISR, however, should be critically analyzed to assess the potential risk of a systemic drug intolerance.

## 6. Expert opinion

The therapeutic potential of ETN for the approved indications is high. Given its molecular structure as rhu fusion protein, the formation of antibodies that affect TNF binding and reduce the therapeutic efficacy is unlikely. In this respect it clearly

differs from therapeutic monoclonal antibodies against TNF- $\alpha$  that may lose efficacy due to the formation of blocking antibodies. The formation of ADAs against the fusion site, however, may induce immune-mediated ISRs that may require a more detailed analysis in select patients. Overall, however, the occurrence of systemic anti-drug reaction is very rare. Assays to define the particular immunoglobulin isotype of the ETN-specific antibodies might help to distinguish IgE- and IgG-mediated ISRs and identify the risk of anaphylactic reactions in those patients with immediate-type ISRs. Given the unique molecular structure of ETN versus monoclonal antibodies, ETN will remain an important drug for chronic inflammatory disorders in the future and should basically not be affected by the frequency of ISRs.

## Bibliography

Papers of special note have been highlighted as either of interest (●) or of considerable interest (●●) to the readers.

1. Tracey D, Klareskog L, Sasso EH, et al. Tumor necrosis factor antagonist mechanisms of action: a comprehensive review. *Pharmacol Ther* 2008;117:244–79
2. Kerensky TA, Gottlieb AB, Yaniv S, Au SC. Etanercept: efficacy and safety for approved indications. *Expert Opin Drug Saf* 2012;11:121–39
3. Murdaca G, Colombo BM, Contini P, Puppo F. Determination of lymphotoxin-alpha levels in patients with psoriatic arthritis undergoing etanercept treatment. *J Interferon Cytokine Res* 2012;32:277–9
4. Murdaca G, Colombo BM, Cagnati P, et al. Update upon efficacy and safety of TNF-alpha inhibitors. *Expert Opin Drug Saf* 2011;11:1–5
5. Leonardi CL, Powers JL, Matheson RT, et al. Etanercept as monotherapy in patients with psoriasis. *N Engl J Med* 2003;349:2014–22
6. Gottlieb AB, Matheson RT, Lowe N, et al. A randomized trial of etanercept as monotherapy for psoriasis. *Arch Dermatol* 2003;139:1627–32
7. Papp KA, Tying S, Lahfa M, et al. A global phase III randomized controlled trial of etanercept in psoriasis: safety, efficacy, and effect of dose reduction. *Br J Dermatol* 2005;152:1304–12
- **Large RCT assessing efficacy and safety of etanercept as monotherapy for psoriasis. The frequency and clinical characteristics of ETN-ISRs as well as frequency of anti-etanercept antibodies**

**and their consequence for treatment continuation were assessed.**

8. Tying S, Gordon KB, Poulin Y, et al. Long-term safety and efficacy of 50 mg of etanercept twice weekly in patients with psoriasis. *Arch Dermatol* 2007;143:719–26
- **Large RCT assessing efficacy and safety of etanercept as monotherapy for psoriasis. The frequency and clinical characteristics of ETN-ISRs as well as the frequency of anti-etanercept antibodies and their consequences for treatment continuation were assessed.**
9. van de Kerkhof PC, Segaert S, Lahfa M, et al. Once weekly administration of etanercept 50 mg is efficacious and well tolerated in patients with moderate-to-severe plaque psoriasis: a randomized controlled trial with open-label extension. *Br J Dermatol* 2008;159:1177–85
10. Paller AS, Siegfried EC, Langley RG, et al. Etanercept treatment for children and adolescents with plaque psoriasis. *N Engl J Med* 2008;358:241–51
11. Weinblatt ME, Kremer JM, Bankhurst AD, et al. A trial of etanercept, a recombinant tumor necrosis factor receptor:fc fusion protein, in patients with rheumatoid arthritis receiving methotrexate. *N Engl J Med* 1999;340:253–9
12. Moreland LW, Schiff MH, Baumgartner SW, et al. Etanercept therapy in rheumatoid arthritis. A randomized, controlled trial. *Ann Intern Med* 1999;130:478–86
13. Bathon JM, Martin RW, Fleischmann RM, et al. A comparison of etanercept and methotrexate in patients

with early rheumatoid arthritis. *N Engl J Med* 2000;343:1586–93

14. Klareskog L, van der Heijde D, de Jager JP, et al. Therapeutic effect of the combination of etanercept and methotrexate compared with each treatment alone in patients with rheumatoid arthritis: double-blind randomised controlled trial. *Lancet* 2004;363:675–81
15. Mease PJ, Kivitz AJ, Burch FX, et al. Etanercept treatment of psoriatic arthritis: safety, efficacy, and effect on disease progression. *Arthritis Rheum* 2004;50:2264–72
16. Zeltser R, Valle L, Tanck C, et al. 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;137:893–9
- **Retrospective analysis of frequency, clinical course and characteristics of ETN-ISRs. A detailed histological and immunophenotypic analysis was performed in order to investigate the potential mechanisms of their development.**
17. Papp KA. The safety of etanercept for the treatment of plaque psoriasis. *Ther Clin Risk Manag* 2007;3:245–58
18. Murphy FT, Enzenauer RJ, Battafarano DF, David-Bajar K. Etanercept-associated injection-site reactions. *Arch Dermatol* 2000;136:556–7
- **Case report on an ETN-ISR containing a detailed histological analysis including direct immunofluorescence staining.**

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## Declaration of interest

JC Prinz has served as a consultant, investigator, speaker or advisory board member for several pharmaceutical companies, including Biogen-Idec (formerly Biogen), Novartis, Wyeth/Pfizer, Merk-Serono (formerly Serono), Essex pharma/MSD, Galderma, Centocor, Abbott, Janssen-Cilag/Janssen-Ortho. A Batycka-Baran was recipient of the Otto-Braun-Falco scholarship.

19. Available from: <http://www.ncbi.nlm.nih.gov/sites/entrez>
20. Wolbink GJ, Aarden LA, Dijkmans BA. Dealing with immunogenicity of biologicals: assessment and clinical relevance. *Curr Opin Rheumatol* 2009;21:211–15
21. Anderson PJ. Tumor necrosis factor inhibitors: clinical implications of their different immunogenicity profiles. *Semin Arthritis Rheum* 2005;34:19–22
- **Discusses the immunogenicity of different Tumor Necrosis Factor Inhibitors.**
22. Keystone EC, Schiff MH, Kremer JM, et al. Once-weekly administration of 50 mg etanercept in patients with active rheumatoid arthritis: results of a multicenter, randomized, double-blind, placebo-controlled trial. *Arthritis Rheum* 2004;50:353–63
23. Dore RK, Mathews S, Schechtman J, et al. The immunogenicity, safety, and efficacy of etanercept liquid administered once weekly in patients with rheumatoid arthritis. *Clin Exp Rheumatol* 2007;25:40–6
24. Lovell DJ, Giannini EH, Reiff A, et al. Etanercept in children with polyarticular juvenile rheumatoid arthritis. *Pediatric Rheumatology Collaborative Study Group. N Engl J Med* 2000;342:763–9
25. de Vries MK, van der Horst-Bruinsma IE, Nurmohamed MT, et al. Immunogenicity does not influence treatment with etanercept in patients with ankylosing spondylitis. *Ann Rheum Dis* 2009;68:531–5
26. Gonzalez-Lopez MA, Martinez-Taboada VM, Gonzalez-Vela MC, et al. Recall injection-site reactions associated with etanercept therapy: report of two new cases with immunohistochemical analysis. *Clin Exp Dermatol* 2007;32:672–4
27. Edwards KR, Mowad CM, Tyler WB. Worsening injection site reactions with continued use of etanercept. *J Drugs Dermatol* 2003;2:184–7
- **Case report on ETN-ISR with a detailed analysis of the potential mechanism of ISRs.**
28. Papadavid E, Makris M, Dalamaga M, et al. Recall injection-site reactions to etanercept in a patient with psoriasis. *Clin Exp Dermatol* 2009;34:414–15
29. Rajakulendran S, Deighton C. Delayed multiple injection site reaction in a rheumatoid arthritis patient treated with etanercept. *Rheumatology (Oxford)* 2004;43:1588–9
30. Winfield H, Lain E, Horn T, Hoskyn J. Eosinophilic cellulitislike reaction to subcutaneous etanercept injection. *Arch Dermatol* 2006;142:218–20
31. Benucci M, Manfredi M, Demoly P, Campi P. Injection site reactions to TNF-alpha blocking agents with positive skin tests. *Allergy* 2008;63:138–9
- **Case report on ETN-ISRs with a precise description of clinical characteristics and course of ETN-ISRs including etanercept skin testing.**
32. Bavbek S, Aydin O, Ataman S, et al. Injection-site reaction to etanercept: role of skin test in the diagnosis of such reaction and successful desensitization. *Allergy* 2011;66:1256–7
- **Case report on an ETN-ISR including etanercept skin testing and a protocol for desensitization.**
33. Pichler WJ. Adverse side-effects to biological agents. *Allergy* 2006;61:912–20
- **Proposal for a classification of immune-mediated adverse events of biologics.**
34. Schellekens H. Immunogenicity of therapeutic proteins. *Nephrol Dial Transplant* 2003;18:1257–9
35. Sauerborn M, Schellekens H. B-1 cells and naturally occurring antibodies: influencing the immunogenicity of recombinant human therapeutic proteins? *Curr Opin Biotechnol* 2009;20:715–21
36. Christen U, Thuerkauf R, Stevens R, Lesslauer W. Immune response to a recombinant human TNFR55-IgG1 fusion protein: auto-antibodies in rheumatoid arthritis (RA) and multiple sclerosis (MS) patients have neither neutralizing nor agonist activities. *Hum Immunol* 1999;60:774–90
37. Galaria NA, Werth VP, Schumacher HR. Leukocytoclastic vasculitis due to etanercept. *J Rheumatol* 2000;27:2041–4
38. Brion PH, Mittal-Henkle A, Kalunian KC. Autoimmune skin rashes associated with etanercept for rheumatoid arthritis. *Ann Intern Med* 1999;131:634
39. Misery L, Perrot JL, Gentil-Perret A, et al. Dermatological complications of etanercept therapy for rheumatoid arthritis. *Br J Dermatol* 2002;146:334–5
40. Silver J, Benacerraf B. Dissociation of T cell helper function and delayed hypersensitivity. *J Immunol* 1974;113:1872–5
41. Nydegger UE. Immune complex pathophysiology. *Ann N Y Acad Sci* 2007;1109:66–83
42. Delves PJ, Martin SJ, Burton DR, Roitt IM. Roitt's essential immunology. Wiley-Blackwell; Oxford: 2011
43. Norman MU, Lister KJ, Yang YH, et al. TNF regulates leukocyte-endothelial cell interactions and microvascular dysfunction during immune complex-mediated inflammation. *Br J Pharmacol* 2005;144:265–74
- **The mechanism of Arthus reactions is being discussed.**
44. Kohl J, Gessner JE. On the role of complement and Fc gamma-receptors in the Arthus reaction. *Mol Immunol* 1999;36:893–903
45. Orito H, Fujimoto M, Ishiura N, et al. Intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 cooperatively contribute to the cutaneous Arthus reaction. *J Leukoc Biol* 2007;81:1197–204
46. Skytta E, Pohjankoski H, Savolainen A. Etanercept and urticaria in patients with juvenile idiopathic arthritis. *Clin Exp Rheumatol* 2000;18:533–4
47. Delves PJ, Roitt IM. Encyclopedia of immunology. Academic Press; London: 1998
48. Jacysyn JF, Abrahamsohn IA, Macedo MS. Modulation of delayed-type hypersensitivity during the time course of immune response to a protein antigen. *Immunology* 2001;102:373–9
49. Askenase PW, Atwood JE. Basophils in tuberculin and "Jones-Mote" delayed reactions of humans. *J Clin Invest* 1976;58:1145–54
- **Differentiates tuberculin and Jones-Mote types of DTH.**
50. Richerson HB, Dvorak HF, Leskowitz S. Cutaneous basophilic hypersensitivity: a new interpretation of the Jones-Mote reaction. *J Immunol* 1969;103:1431–4
51. Kopf M, Abel B, Gallimore A, et al. Complement component C3 promotes T-cell priming and lung migration to

control acute influenza virus infection.

Nat Med 2002;8:373–8

- **Identifies the relevance of complement activation for T-cell recruitment.**

52. Borrás-Blasco J, Gracia-Perez A, Rosique-Robles JD, et al. Urticaria due to etanercept in a patient with psoriatic arthritis. South Med J 2009;102:304–5

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