# Anti-CD20 (rituximab) treatment improves atopic eczema

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**Background: Atopic eczema (AE) is a chronic inflammatory skin disorder characterized by eczematous skin lesions, pruritus, and typical histopathologic features.**
**Objective: We asked whether depletion of B cells by monoclonal anti-CD20 antibody therapy (rituximab) would improve severe AE.**
**Methods: Six patients (4 women and 2 men) with severe AE received 2 intravenous applications of rituximab, each 1000 mg, 2 weeks apart. To evaluate the efficacy of rituximab, we monitored clinical parameters (eczema area and severity index, pruritus), total and allergen-specific IgE levels, skin histology, and inflammatory cells and cytokine expression in the skin and peripheral blood before and after therapy (ClinicalTrials.gov Identifier: NCT00267826).**
**Results: All patients showed an improvement of their skin symptoms within 4 to 8 weeks. The eczema area and severity index significantly decreased (before therapy, 29.4 ± 4.3; week 8, 8.4 ± 3.6; $P < .001$). Histologic alterations such as spongiosis, acanthosis, and dermal infiltrate, including T and B cell numbers, also dramatically improved. However, whereas blood B cells were below detectable levels as a consequence of rituximab administration, skin B cells were reduced by approximately 50% only. Expression of IL-5 and IL-13 was reduced after therapy. Moreover, whereas allergen-specific IgE levels were not altered, we observed a slight reduction in total IgE concentrations in blood.**
**Conclusions: B cells play a major role in AE pathogenesis. Treatment with an anti-CD20 antibody leads to an impressive improvement of AE in patients with severe disease.**
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***Key words:*** *Atopic dermatitis, B cells, biologics, cytokines, inflammation, rituximab, T cells*

*Abbreviations used*
AE: Atopic eczema
EASI: Eczema area and severity index
ECP: Eosinophil cationic protein

Atopic eczema (AE) is a chronic inflammatory skin disorder based on a genetic predisposition and triggered by environmental factors presenting with erythematous, oozing or scaling lesions, and severe pruritus.[1] The typical histopathologic findings are acanthosis and spongiosis of the epidermis, as well as a perivascular infiltrate in the dermis, consisting predominantly of T cells,[2] but variable numbers of B cells, mast cells, and eosinophils are also present.[3-5] In about 80% of patients with AE, elevated levels of total IgE and/or specific IgE to environmental allergens are found.[6] Furthermore, Langerhans cells of AE skin bear the high-affinity IgE receptor on their surface, thus providing the link between environmental allergen exposure and T-cell activation.[7] These observations point to the possibility that B cells play a role in AE pathogenesis.

Rituximab is a chimeric monoclonal anti-CD20 antibody, originally developed for the therapy of B-cell malignancies, that eliminates B cells[8] by inducing antibody-dependent cell-mediated cytotoxicity, complement-dependent toxicity, or apoptosis.[9] CD20 is expressed by pre-B cells and mature B cells, but not plasma cells.[10] Recent studies reported the efficacy of rituximab in the treatment of autoimmune diseases, such as rheumatoid arthritis,[11-13] SLE,[14,15] and pemphigus vulgaris.[16-20] The efficacy of rituximab in these clinical studies could not simply be explained by a reduction in autoantibody titers, because this was often not the case. Instead, it was suggested that the loss of the antigen-presenting and immunomodulatory functions of B cells might be responsible for the beneficial clinical effects.[21]

Because it was likely that, besides T cells, B cells play a role in AE pathogenesis, we initiated a pilot study to investigate the clinical efficacy and immunopharmacologic effects of rituximab in AE. In addition to clinical parameters, we monitored inflammatory cells and cytokines in peripheral blood and skin lesions.

## METHODS

### Patients

In this investigator-initiated, open-label pilot study, 6 patients with severe AE according to the criteria of Hanifin and Rajka[22] (4 women, 2 men; age, 19-63 years; average, 39 ± 7 years) were enrolled between October 2005 and April 2006. All patients had not adequately responded to topical corticosteroid and/or calcineurin inhibitor therapy and were candidates for, or had previously received, systemic treatment. Patient characteristics are given in Table I. The study was approved by the ethics committee of the Canton Bern. Written informed consent was obtained from all patients before participation in the study. All patients had a 2-week washout of all topical therapy except corticosteroids and a 4-week washout of systemic treatment except antihistamines. Two weeks before rituximab administration and during the study period, the treatment was restricted to moderately potent topical corticosteroids (mometasone, halometasone) and antihistamines (hydroxycine, cetericine) as needed. Complement deficiency, immunoglobulin deficiencies, and severe infections had been ruled out before rituximab treatment. The study design is shown in Fig 1. Rituximab (MabThera; Roche Pharma [Schweiz] AG, Reinach, Switzerland) was administered twice, each 1000 mg intravenous, 2 weeks apart. To avoid intolerance reactions, 1000 mg paracetamol and 2 mg clemastinum were given before each

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**TABLE I.** Patient characteristics

| Patient no. | Sex, age (y) | Duration of AE | Grading of AE* | EASI at baseline | Total IgE (kU/L) | Specific IgE to inhalative allergens† (kU/L) | Specific IgE‡ | Skin prick test‡ |
|---|---|---|---|---|---|---|---|---|
| 1 | Male, 48 | 20 | 9 | 38.0 | 4750 | 89.9 | B,G,C, HDM | B,G,C, HDM |
| 2 | Female, 27 | 13 | 8 | 32.0 | 1874 | 93.1 | G,C, HDM | G,C, HDM |
| 3 | Female, 49 | 47 | 9 | 27.0 | 3010 | 78.4 | G,C | G,C |
| 4 | Female, 19 | 7 | 8 | 17.0 | 471 | 26.9 | B,G | B,G |
| 5 | Male, 26 | 22 | 9 | 43.5 | 2275 | >100 | B,G,C, HDM | Not done |
| 6 | Female, 63 | 20 | 8 | 18.0 | 494 | 16.8 | HDM | HDM |

*Grading of long-term severity according to Langeland and Rajka (1-3 = mild AE; 7-9 = severe AE).
†Screening test of 8 common inhalative allergens (house dust mite [*Dermatophagoides pteronyssinus*], cat and dog danders, mold [*Cladosporium herbarum*], and pollens of birch, grass, mugwort, and rye).
‡Test for pollens of birch *(B)* and grass *(G)*, cat dander *(C)*, and house dust mites *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae (HDM).*

infusion. Punch biopsies were taken from lesional skin before and from the same or symmetrical areas 6 weeks after therapy. All 6 patients finished the study including the 22-week observation period after therapy.

## Clinical parameters

To evaluate the clinical efficacy of rituximab therapy, the eczema area and severity index (EASI),[23] a pruritus score (0, no; 1, mild; 2, moderate; 3, severe), the use of concomitant therapy for AE such as topical corticosteroids and antihistamines, and adverse events were monitored at each visit.

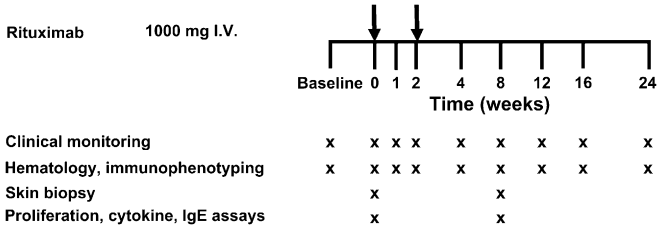


**FIG 1.** Study design. *I.V.,* Intravenous.

## Peripheral blood analysis

To monitor platelet, leukocyte, and differential counts, an automatic blood count analysis system (Sysmex KX-21, Sysmex Corp, Kobe, Japan; Digitana SA, Yverdon-les-Bains, Switzerland) was used. To determine lymphocyte subsets, blood was analyzed by standard flow-cytometric techniques using a FACS Calibur (BD Biosciences, Basel, Switzerland).[24,25] Total and specific IgE levels were measured with the UniCAP system (Phadia, Uppsala, Sweden).

PBMCs were separated from peripheral blood by Ficoll-Hypaque density gradient centrifugation.[26,27] The cells were suspended at $1 \times 10^6$/mL in complete culture medium (RPMI 1640 supplemented with 2 mmol/L L-glutamine, 200 IU/mL penicillin, 100 μg/mL streptomycin, and 10% FBS; all from Life Technologies, Basel, Switzerland) and cultured in the presence and absence of phytohemagglutinin for 24 hours. The supernatants were harvested and frozen at –80°C until cytokine analysis. IL-2, IL-4, IL-5, and TNF-α were measured using a Cytometric Bead Array assay (BD Biosciences). IL-13 levels were determined with the Quantikine ELISA kit (R&D Systems, Abingdon, United Kingdom).

## Skin histology

Sections of 6 μm of the paraformaldehyde-fixed, paraffin-embedded skin biopsies were stained with hematoxylin and eosin and examined by light microscopy in a blind fashion (Axiovert 35; Carl Zeiss, Heidelberg, Germany). To assess the extent of hyperkeratosis, acanthosis, spongiosis, and dermal infiltrate, a semiquantitative score (0, none; 1, mild; 2, moderate; 3, severe) was applied.[4]

## Immunohistologic examination

Immunofluorescence staining was undertaken on 4-μm sections of paraformaldehyde-fixed and paraffin-embedded skin biopsies. To identify the inflammatory cell pattern in the dermis, sections were treated with monoclonal antibodies against CD4 ($T_H$ cells) and CD8 (T effector cells) purchased from Serotec (Düsseldorf, Germany), against CD20 and CD21 (B cells) from Biogenesis (Poole, United Kingdom [UK]), against eosinophil cationic protein (ECP; eosinophils) from Pharmacia & Upjohn Diagnostics AB (Uppsala, Sweden), and against tryptase (mast cells), CD1a (epidermal dendritic cells), and CD138 (plasma cells; all from Dako, Baar, Switzerland) at 4°C overnight. To analyze cytokine expression by T cells, in addition to the CD4 or CD8 antibodies, antibodies recognizing human IL-5 and IL-13 (both from Santa Cruz Biotechnology, Santa Cruz, Calif) as well as against IL-10 and IFN-γ (both from R&D Systems) were used. Antibody binding was detected with appropriate Alexa Fluor 488–conjugated (Invitrogen, Paisley, United Kingdom) and/or indocarbocyanine (Cy3)–conjugated (Jackson ImmunoResearch, Suffolk, United Kingdom) secondary antibodies. Mouse $IgG_1$ and rabbit IgG negative control antibodies (both from Dako) and normal goat IgG (R&D Systems) served as negative controls. Immunofluorescence staining was evaluated by 2 independent investigators using a confocal laser scanning microscope (LSM 510; Carl Zeiss) equipped with argon and helium-neon lasers. Positive cells were counted in 10 consecutive fields, which were 0.1 $mm^2$, at a magnification of ×1000.

## Real-time PCR

Total RNA was extracted from fifty 10-μm sections by using the RNeasy Fibrous Tissue Kit (Qiagen, Basel, Switzerland). Contaminating DNA was removed with DNase (Qiagen). Total RNA yield and quality were determined by the NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, De) and Agilent 2100 bioanalyzer (Agilent Technologies, Palo Alto, Calif). cDNA was prepared with Powerscript RT (BD Biosiences) and random primers (Promega, Madison, Wis) according to the manufacturer's instructions. PCR was performed by using TaqMan Gene Expression Assays (Applied Biosystems, Foster City, Calif). PCR primers and probes were purchased as premade assays on demand spanning exon-exon borders for IFN-γ, IL-5, IL-10, IL-13, and 4 control genes (ribosomal 18S RNA, hypoxanthine phosphoribosyltransferase 1, β2 microglobulin, glyceraldehyde-3-phosphate dehydrogenase). By using geNorm software (PrimerDesign Ltd, Southampton, UK), hypoxanthine phosphoribosyltransferase 1 was determined as the most stable expressed control gene and further used as an endogenous reference gene.[28] Amplification reactions were performed in a final volume of 20 μL with 1 μL cDNA, 1 set each of TaqMan Universal PCR Master Mix and Assay-on-Demand Gene Expression Assay Mix using the 7300 Real Time PCR System (both Applied Biosystems).