Journal of Dermatological Science 76 (2014) 96–103

Contents lists available at ScienceDirect

Journal of Dermatological Science

journal homepage: www.jdsjournal.com

Antibody-mediated delivery of interleukin 4 to the neo-vasculature reduces chronic skin inflammation

Teresa Hemmerle [a], Silvana Zgraggen [a], Mattia Matasci [b], Cornelia Halin [a], Michael Detmar [a], Dario Neri [a,*]

[a] Department of Chemistry and Applied Biosciences, ETH Zürich, Wolfgang-Pauli-Strasse 10, CH-8093 Zurich, Switzerland
[b] Philochem AG, Libernstrasse 3, CH-8093 Otelfingen, Switzerland

ARTICLE INFO

Article history:
Received 11 February 2014
Received in revised form 1 July 2014
Accepted 27 July 2014

Keywords:
Psoriasis
Chronic skin inflammation
Interleukin 4
Antibody-cytokine fusion protein
Fibronectin

ABSTRACT

Background: The antibody-mediated delivery of cytokines (''immunocytokines'') to sites of pathological angiogenesis represents an attractive strategy for the development of innovative biopharmaceuticals, capable of modulating the activity of the immune system in cancer and in chronic inflammatory conditions.
Objective: Recombinant IL4 has previously been shown to be therapeutically active in patients with psoriasis. The antibody-mediated delivery of this cytokine to sites of chronic skin inflammatory conditions should lead to an improved potency and selectivity, compared to non-targeted IL4.
Methods: The therapeutic activity of F8-IL4, a fusion protein of the F8 antibody (specific to the alternatively-spliced EDA domain of fibronectin) with murine IL4, was investigated in three immunocompetent mouse models of skin inflammation: two induced by the TLR7/8 ligand imiquimod (in Balb/c and C57BL/6) and one mediated by the over-expression of VEGF-A.
Results: The EDA domain of fibronectin, a marker for angiogenesis, is expressed in the inflamed skin in all three models and F8-IL4 selectively localized to inflamed skin lesions following intravenous administration. The F8-IL4 fusion protein mediated a therapeutic benefit, which was superior to the one of a non-targeted version of IL4 and led to increased levels of key regulatory cytokines (including IL5, IL10, IL13, and IL27) in the inflamed skin, while IL2 levels were not affected in all treatment groups. A murine version of etanercept and a murine anti-IL17 antibody were used as positive control in the therapy experiments.
Conclusion: Skin inflammatory lesions can be selectively targeted using anti-EDA antibody–cytokine fusion proteins and the pharmacodelivery of IL4 confers a therapeutic benefit by shifting the cytokine balance.

1. Introduction

Psoriasis is a chronic inflammatory autoimmune disease of the skin and small joints that impairs the quality of life of 1–2% of the human population. The disease results from a combination of genetic and environmental factors, leading to a thickening of the epidermis and to the increased proliferation of keratinocytes [1–3].

The treatments of psoriasis can be divided in three main categories of pharmacological intervention: topical therapy, phototherapy and pharmacotherapy with immunomodulatory drugs. The topical treatment for mild-to-moderate psoriasis consists of topical corticosteroids, vitamin D analogs, anthralin, topical retinoids, calcineurin inhibitors, salicylic acid, coal tar and moisturizers. For more severe psoriasis, the topical treatment is combined with either phototherapy using natural sunlight or artificial ultraviolet light or with oral drugs, such as retinoids, methotrexate, cyclosporine, hydroxyurea, thioguanine. More recently, several immunomodulatory biologicals [such as alefacept (Amevive™), etanercept (Enbrel™) infliximab (Remicade™) and ustekinumab (Stelara™)], which are administered *via* the intravenous, intramuscular or subcutaneous route, have been approved

Abbreviations: IL4, interleukin 4; IMQ, imiquimod; PASI, psoriasis area severity index; TNFR, tumor necrosis factor receptor; TLR7/8, toll-like receptor 7/8; SIP, small immune protein.

* Corresponding author. Tel.: +41 44 6337401.
E-mail address: neri@pharma.ethz.ch (D. Neri).

http://dx.doi.org/10.1016/j.jdermsci.2014.07.012
0923-1811/

for the treatment of moderate-to-severe psoriasis [1,3,4]. Several emerging products are currently in advanced clinical development programs, including antibodies that target IL23 (p19 subunit) or IL17, as well as JAK3 low molecular weight inhibitors, which prevent the signaling of common gamma-chain cytokines (IL2, IL4, IL7, IL9, IL15 and IL21) [2]. Indeed, IL17 blockers are exhibiting impressive therapeutic results in psoriasis patients, with up to 82% of patients enjoying a PASI75 benefit by week 12 [5]. The anti-IL17A antibody secukinumab is currently filed for marketing authorization in Japan, the United States and the European Union for the treatment of psoriasis and psoriatic arthritis [2,6].

The histological changes in psoriatic lesions are accompanied by the formation of hyperplastic dermal blood vessels and by a rich infiltrate of immune cells in the skin [1,7]. High levels of interferon-gamma, as well as low levels of IL10 and IL4, indicate that a T_H1 immunomodulatory mechanism may play a role in the disease. As a consequence, pharmaceutical interventions, aiming at a shift from a T_H1 to a T_H2 environment in the inflamed skin, have been proposed as a therapeutic strategy for the treatment of psoriasis [8,9].

Recombinant IL4 was originally investigated as immune modulator with anti-cancer potential, but despite promising preclinical results [10–13], a lack of efficacy at the doses used in the clinical trials (up to 5 μg/kg/day) prevented further developments in oncology [14–16]. Interestingly, some cancer patients with concomitant psoriasis were reported to benefit from IL4 treatment [17]. These findings were supported by several *in vitro* and *in vivo* studies, which confirmed the ability of IL4 to mediate a potent induction of T_H2 cell differentiation and promising therapeutic results [18–20].

While cytokines may display a potent therapeutic activity in preclinical models of cancer and other conditions, their clinical use is often limited by severe systemic toxicities that prevent a dose escalation to therapeutically active regimes. The antibody-mediated pharmacodelivery of cytokines to the site of disease may help to increase therapeutic activity, while sparing normal tissues. Several antibody–cytokine fusion proteins (“immunocytokines”) are in development for cancer therapy indications [21,22]. More recently, IL10-based immunosuppressive antibody–cytokine fusion proteins have been proposed for the therapy of chronic inflammatory conditions [23–27] and one IL10-based immunocytokine is currently being investigated in Phase I studies in patients with rheumatoid arthritis [28]. It would be desirable to develop immunocytokines active both against cancer and chronic inflammatory conditions, since these products would allow the treatment of polymorbid patients and may be associated with a benign safety profile.

The F8 antibody, specific to the alternatively spliced extra-domain A (EDA) of fibronectin, is particularly suited for *in vivo* pharmacodelivery applications in cancer and in chronic inflammation, as it recognizes its cognate antigen in virtually all cancer types and in many inflammatory conditions, while being undetectable in the majority of healthy tissues, exception made for placenta, the endometrium in the proliferative phase and some vessels in the ovaries [25,29–31]. EDA has only three amino acid mutations from mouse to man and the F8 antibody binds to both proteins with identical affinity, thus facilitating preclinical studies in immunocompetent mouse models and clinical development activities [31]. We have recently constructed an antibody–cytokine fusion protein based on F8 and murine IL4 (“F8-IL4”), which has exhibited a potent anti-cancer activity in all syngeneic immunocompetent mouse models of cancer tested so far [32].

In this article, we describe the targeting and therapeutic activity of F8-IL4 in acute and chronic mouse models of skin inflammation, based on the over-expression of VEGF-A in the mouse skin after challenge with a contact sensitizer (contact-hypersensitivity model) or based on the topical applications of imiquimod (IMQ). F8-IL4 was more active than an IL4-based immunocytokine specific to hen egg lysozyme (thus serving as an non-targeted, negative control) and displayed a therapeutic action comparable to the one observed with a murine analog of etanercept [23]. Analysis of cytokine levels in the inflamed skin revealed that F8-IL4 substantially alters the expression of key immunomodulatory cytokines.

2. Materials and methods

2.1. Proteins and animals

The fusion proteins were expressed from stable monoclonal cell lines in CHO cells as previously reported: briefly, cells that were stably transfected with SIP(KSF), murine TNFR-Fc or F8-IL4 respectively KSF-IL4 [32] were grown in suspension in Power-CHO-2CD medium (Lonza). The proteins were purified from cell culture supernatant by protein A affinity chromatography and analyzed by SDS-PAGE and size exclusion chromatography (Superdex200 10/300GL; GE Healthcare). SIP(F8) [31] was provided by Philogen SpA (Siena, Italy) and anti-mouse IL17A antibody was bought from Bio X Cell (clone 17F3). Female C57BL/6 and FVB mice were obtained from Charles River (Sulfzfeld, Germany), Balb/c mice from Janvier (Le-Genest, France) and K14-VEGF-A transgenic mice were bred in house at the ETH Zürich.

2.2. Immunofluorescence analysis

For the detection of the antigen EDA in the mouse models, frozen sections of murine psoriatic ear skin (10 μm) were fixed in ice-cold acetone and stained with biotinylated SIP(F8) and SIP(KSF) (specific to hen egg lysozyme; used as negative control). Rat anti-CD31 (BD Bioscience) was used for vascular staining. Bound antibodies were detected using streptavidin-AlexaFluor488 (Invitrogen) and donkey anti-rat IgG-AlexaFluor594 (Invitrogen). Slides were mounted with fluorescent mounting medium (Dako) and analyzed with an Axioskop2 mot plus microscope (Zeiss).

2.3. Biodistribution and autoradiography experiments

The *in vivo* targeting performance of SIP(F8) respectively SIP(KSF) and F8-IL4 respectively KSF-IL4 were evaluated by quantitative biodistribution analysis as described previously [33]. Briefly, radioiodiated protein was injected into the lateral tail vein of mice. Mice were sacrificed 24 h after injection, organs were excised and radioactivity was counted in Cobra gamma counter (Packard). Radioactivity was expressed as percentage of the injected dose per gram of tissue (%ID/g). For autoradiography experiments, ears were exposed for 48 h and read with a phosphor-imager (BAS5000, Fujifilm).

2.4. IMQ-induced skin inflammation

C57BL/6 or Balb/c mice were treated on each side of both ears with 5 mg Aldara cream containing 0.25 mg imiquimod (IMQ) (3M Health Care Ltd) for five consecutive days and on day 7 (Fig. 2). The TLR7/8 ligand IMQ is a potent immune activator and the resulting inflammation depends on the IL-23/IL-17 axis, namely Th17 cells [34]. Ear thickness was measured repeatedly with a caliper. For the study of a chronic state of inflammation, IMQ was additionally applied on day 9 and 11, leading to maintenance of the disease (Fig. 2). Experiments were performed in agreement with the Swiss regulations and under a project license granted by the Veterinäramt des Kantons Zürich (117/2011).