

Targeting Interleukin-15 in Patients With Rheumatoid Arthritis

A Proof-of-Concept Study

Bo Baslund,¹ Niels Tvede,¹ Bente Danneskiold-Samsøe,² Per Larsson,³ Gabriel Panayi,⁴
Joergen Petersen,⁵ Lars J. Petersen,⁵ Frank J. M. Beurskens,⁶ Janine Schuurman,⁶
Jan G. J. van de Winkel,⁶ Paul W. H. I. Parren,⁶ J. Alastair Gracie,⁷ Sarah Jongbloed,⁷
Foo Y. Liew,⁷ and Iain B. McInnes⁷

Objective. Interleukin-15 (IL-15) is a proinflammatory, innate response cytokine that mediates pleiotropic effector function in rheumatoid arthritis (RA) inflammatory synovitis. Our objective was to study the ability of HuMax-IL15, a human IgG1 anti-IL-15 monoclonal antibody, to neutralize exogenous and endogenous IL-15 activity in vitro and to perform a phase I–II dose-escalation trial with HuMax-IL15 in patients with active RA.

Methods. Mononuclear cells from blood and synovial fluid (SF) of RA patients were isolated and cultured in vitro under experimental conditions involving the addition of HuMax-IL15. HuMax-IL15 was administered to 30 RA patients who received no other disease-modifying antirheumatic drugs in a 12-week, dose-

ascending, placebo-controlled, double-blind, phase I–II proof-of-concept study.

Results. In vitro studies showed that HuMax-IL15 suppressed proliferation and induced apoptosis in an IL-15-dependent cell line, BDB2, and was capable of suppressing the release of interferon- γ by synovial fluid mononuclear cell (SFMC) cultures induced by exogenous IL-15. Furthermore, HuMax-IL15 F(ab')₂ fragments suppressed exogenous IL-15-induced CD69 expression in RA peripheral blood mononuclear cells and SFMCs, which indicates that HuMax-IL15 can specifically neutralize several biologic effects of IL-15 in synovial tissue in vitro. In a phase I–II clinical trial, HuMax-IL15 was well tolerated clinically, with no significant effects on T lymphocyte subset and natural killer cell numbers. Substantial improvements in disease activity were observed according to the American College of Rheumatology criteria for 20% improvement (63% of patients), 50% improvement (38%), and 70% improvement (25%).

Conclusion. These clinical data suggest for the first time that IL-15 could represent a novel therapeutic target in RA.

Biologic agents that target tumor necrosis factor α (TNF α) provide substantial clinical benefit in the treatment of several autoimmune diseases, particularly rheumatoid arthritis (RA). However, partial or non-clinical responders to TNF blockade (i.e., failure to achieve a 50% improvement in disease activity according to the American College of Rheumatology criteria [ACR50]) (1) comprise approximately half of recipients, and considerable unmet clinical need (2–4) remains. As such, novel therapeutic targets must be identified. Cyto-

Supported by Genmab, Copenhagen, Denmark.

¹Bo Baslund, MD, PhD, Niels Tvede, MD: Rigshospitalet, Copenhagen, Denmark; ²Bente Danneskiold-Samsøe, MD: Frederiksberg Hospital, Frederiksberg, Denmark; ³Per Larsson, MD: Karolinska Sjukhuset, Stockholm, Sweden; ⁴Gabriel Panayi, MD: Guy's Hospital, London, UK; ⁵Joergen Petersen, MD, DMSc, Lars J. Petersen, MD: Genmab, Copenhagen, Denmark; ⁶Frank J. M. Beurskens, PhD, Janine Schuurman, PhD, Jan G. J. van de Winkel, PhD, Paul W. H. I. Parren, PhD: Genmab, Utrecht, The Netherlands; ⁷J. Alastair Gracie, PhD, Sarah Jongbloed, BSc, Foo Y. Liew, PhD, Iain B. McInnes, MD: University of Glasgow, Glasgow, UK.

Drs. Baslund and Panayi have received consulting fees of less than \$10,000 from Genmab concerning the use of HuMax-IL15 in rheumatoid arthritis. Drs. Petersen, Beurskens, Schuurman, van de Winkel, and Parren own Genmab warrants and/or stocks and have a patent on IL-15 (patents W003/017935 and W02004/076620). Dr. McInnes has received consulting fees of less than \$10,000 from Genmab concerning the use of HuMax-IL15 in rheumatoid arthritis.

Address correspondence and reprint requests to Iain B. McInnes, MD, Centre for Rheumatic Diseases, Glasgow Royal Infirmary, 10 Alexandra Parade, Glasgow G31 2ER, UK. E-mail: i.b.mcinnis@clinmed.gla.ac.uk.

Submitted for publication November 20, 2004; accepted in revised form May 24, 2005.

kines that function in parallel with or upstream from TNF α in inflammatory cascades represent theoretically attractive targets.

Interleukin-15 (IL-15) is a 15-kd, 4 α -helical innate response cytokine that exhibits broad functional pleiotropy by binding to a heterotrimeric IL-15 receptor (IL-15R α , IL-15/2R β , and common γ -chain) (5–8). As such, its signaling effector pathways are distinct from those induced via TNF α . Reported bioactivities include the capacity to recruit and activate T cells, maintain T cell memory, activate neutrophils, and retard apoptosis of fibroblast-like synoviocytes (FLS) and endothelial cells (9–11). Data from *in vitro* studies and studies of IL-15-transgenic and IL-15-deficient mice reveal a significant role in natural killer (NK) cell ontogeny and effector function (12,13). We and others previously demonstrated IL-15 expression on macrophages, endothelial cells, and FLS in RA synovial tissue, where it can exert broad functional effects in chronic synovitis (14–21). IL-15 promotes synovial cytokine (including TNF α) and chemokine release directly via effects on synovial T cells and indirectly through maintenance of cytokine-activated T cell cognate interactions with synovial macrophages (14,22,23). IL-15 and TNF promote expression of NKG2D on CD4 $^{+}$, CD28 $^{-}$ RA T cells, which renders them susceptible to synovial expression of macrophage inhibitory cytokine ligands of NKG2D, thus providing a potential route to enhanced autoreactivity (24). We previously neutralized IL-15 in murine collagen-induced arthritis (CIA) using recombinant soluble IL-15 receptor α (sIL-15R α) and demonstrated substantial amelioration of inflammation and articular destruction (25).

HuMax-IL15 is a high-affinity, fully human IgG1 anti-IL-15 monoclonal antibody generated in human Ig-transgenic mice (26). HuMax-IL15 neutralizes IL-15 biologic activity by a unique mechanism that was recently described (27). It binds to receptor-bound IL-15 at the site required for interaction with the γ -chain and thus frustrates signaling. Since IL-15 is expressed in RA synovial tissue, possesses plausible bioactivities, and is functionally implicated in CIA, we explored its clinical therapeutic potential in RA using this novel anti-IL-15 antibody *in vitro* and *in vivo* in a proof-of-concept study.

PATIENTS AND METHODS

Patients and materials. Peripheral blood (PB) was obtained from healthy volunteers and from RA patients after informed consent was provided. Samples were harvested into heparinized (Leo Laboratories, Prince Risborough, UK) vacu-

ainers and processed immediately. Synovial fluid (SF) was obtained from patients being treated at the Center for Rheumatic Diseases (Glasgow, UK). It was harvested into heparinized containers and processed immediately. Therapeutic aspiration was also performed.

BDB2 cells, an IL-15-dependent, human T cell line, were a kind gift of Dr. John Campbell, University of Glasgow. Synthesis of HuMax-IL15 has been described previously (25). HuMax-IL15 (146B7, AMG714) (27), IgG control (huIgG), and F(ab') $_2$ fragments were provided by Genmab (Utrecht, The Netherlands). Human sIL-15R α was synthesized as previously described (28). Recombinant cytokines were purchased from R&D Systems (Oxford, UK).

Cell cultures. PB and SF mononuclear cell (PBMC and SFMC) preparations were obtained by density-gradient centrifugation (Lymphoprep; Sigma, Poole, UK), washed 3 times in cold phosphate buffered saline, and cultured at 1×10^6 cells/ml in complete RPMI (100 IU/ml penicillin, 100 μ g/ml streptomycin) with 10% fetal calf serum (Life Technologies, Paisley, UK). To examine cytokine release, recombinant IL-15 or IL-12, HuMax-IL15, or control antibodies were added for the duration of the culture, then supernatants were harvested and frozen at -20°C until measured using enzyme-linked immunosorbent assay (ELISA; R&D Biosystems, Oxford, UK). BDB2 cells (1×10^6 /ml) were cultured, with or without IL-15, with HuMax-IL15 F(ab') $_2$, IgG F(ab') $_2$, or sIL-15R α control, and apoptotic cells were counted at 24 hours with annexin V-propidium iodide using a fluorescence-activated cell sorter (FACSCalibur; Becton Dickinson, Cowley, UK). BDB2 proliferation after 24 hours was measured by determining ^3H -labeled thymidine uptake (Amersham Pharmacia Biotech, Little Chalfont, UK) in the final 6 hours of culture. To examine CD69 expression, RA PBMCs or SFMCs (1×10^6 /ml) were rested overnight, then incubated for 24 hours with or without IL-15 (10 ng/ml) in the presence or absence of HuMax-IL15 F(ab') $_2$ fragments or control antibody, prior to fluorescence-activated cell sorting, as previously described (27). Briefly, lymphocytes derived from culture were gated using forward and side scatter and detected using fluorescein isothiocyanate-conjugated anti-CD69 antibody (Becton Dickinson). Isotype control antibodies were included throughout.

Phase I-II clinical trial design. The phase I-II clinical trial was conducted subject to approval from relevant national and local ethical committees and institutional review boards, as required. Patients were recruited in Denmark, Sweden, and the United Kingdom. All patients provided informed consent prior to entry. HuMax-IL15 was administered in dose-ascending order (0.15, 0.5, 1, 2, 4, and 8 mg/kg) to 6 cohorts of 5 patients (4:1 active:placebo). Patients received no disease-modifying antirheumatic drugs (DMARDs) starting 28 days before the study began, but were allowed to continue any current nonsteroidal antiinflammatory drugs and corticosteroids (at a dosage of <10 mg/day) throughout. Patients with active disease (≥ 7 tender and ≥ 4 swollen joints, erythrocyte sedimentation rate [ESR] >28 mm/hour, or C-reactive protein [CRP] >10 mg/dl) were randomized to receive HuMax-IL15 or placebo by double-blind, single subcutaneous infusion (injection if <4 ml), and were followed up for 28 days. In the absence of dose-limiting toxicity by day 28, all patients received 4 additional doses of HuMax-IL15 at weekly intervals by open-label extension. The 0.15 mg/kg cohort was not extended into

repeat dosing, and the 8 mg/kg cohort continued on 4 mg/kg. Patients were followed up for a further 28 days after the last dose.

Evidence of clinical or laboratory adverse events was collected throughout, together with estimates of RA clinical disease activity. These measures included tender joint count, swollen joint count, early morning stiffness, pain score (visual analog scale), patient global assessment, physician global assessment, Health Assessment Questionnaire (disability measure) (29), and levels of acute-phase reactants (ESR and CRP). From these data, American College of Rheumatology responses (20%, 50%, or 70% improvement from baseline by week 9) were calculated as previously described (1). Clinical chemistry studies, hematology studies (hemoglobin level, white blood cell count, and platelet count), and an initial pregnancy test were performed in a central laboratory (Nova Medical Medi-Lab, Copenhagen, Denmark). Fluorescence-activated cell sorting for leukocyte subsets was performed at the outset and weekly thereafter except at weeks 3 and 11. Markers assessed included CD2, CD3, CD4, CD8, CD16, CD56, CD20, CD45RO, CD45RA, and CD64. The presence of human anti-human antibodies (against HuMax-IL15) was determined by ELISA (Genmab).

RESULTS

In vitro activities of HuMax-IL15. First, we characterized the bioactivities of HuMax-IL15 in vitro. The addition of F(ab')₂ fragments of HuMax-IL15 (to obviate Fc interactions), but not irrelevant IgG F(ab')₂ fragments, to the IL-15-dependent T cell line BDB2 suppressed proliferation and induced apoptosis (Figures 1a and b). It was previously demonstrated that IL-15 operates in synergy with IL-12 to promote synovial tissue cytokine production, particularly interferon- γ (IFN γ) (4). IFN γ released by SFMC cultures in response to IL-15 and IL-12 stimulation was neutralized by coincident addition of 10 μ g/ml HuMax-IL15 F(ab')₂ but not the huIgG F(ab')₂ control (Figure 1c), which showed that HuMax-IL15 can neutralize exogenous IL-15 activity in the synovial environment. We and others have also shown that IL-15 enhances CD69 expression on PB and SF T cells. CD69 is, in turn, implicated in cognate interactions between synovial T cells and macrophages (15,30). RA PBMCs or SFMCs were cocultured for 24 hours with IL-15 in the presence or absence of HuMax-IL15 F(ab')₂ or huIgG1 F(ab')₂ fragments and then CD69 was measured by flow cytometry. As expected, HuMax-IL15 F(ab')₂ suppressed exogenous IL-15-induced CD69 expression in RA PBMCs and SFMCs (Figure 2a). In addition, CD3+ T cell subsets expressing CD69 a priori were significantly reduced by HuMax-IL15 if exogenous IL-15 was not added (Figure 2b), suggesting that endogenous IL-15 activity was also neu-

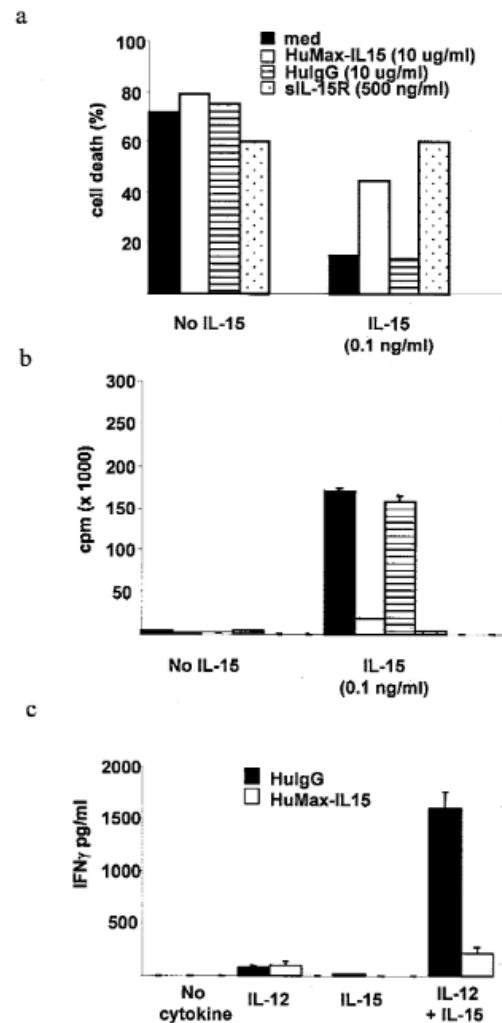


Figure 1. Effects of HuMax-IL15 (10 μ g/ml) on apoptotic cell rescue, proliferation, and cytokine production. **a**, BDB2 cells were cultured, with or without interleukin-15 (IL-15), with HuMax-IL15 F(ab')₂ or human IgG (huIgG) F(ab')₂ negative control, and apoptotic cells were enumerated at 24 hours by fluorescence-activated cell sorting using annexin V-propidium iodide. Soluble IL-15 receptor α (siL-15R α) was used as a positive control. **b**, In parallel experiments, BDB2 cells were cultured for 24 hours and proliferation was measured by determining ³H-labeled thymidine uptake in the last 6 hours of culture. **c**, Rheumatoid arthritis (RA) synovial fluid mononuclear cells were incubated for 16 hours in RPMI/10% fetal calf serum with the cytokines shown (2 ng/ml IL-12, 10 ng/ml IL-15), with or without HuMax-IL15 F(ab')₂, and interferon- γ (IFN γ) levels were measured by enzyme-linked immunosorbent assay. Similar data were obtained in cells from 4 RA patients. Cytokine production was not inhibited by huIgG F(ab')₂ in control cultures. Values are the mean and SEM of triplicate cultures. med = medium.

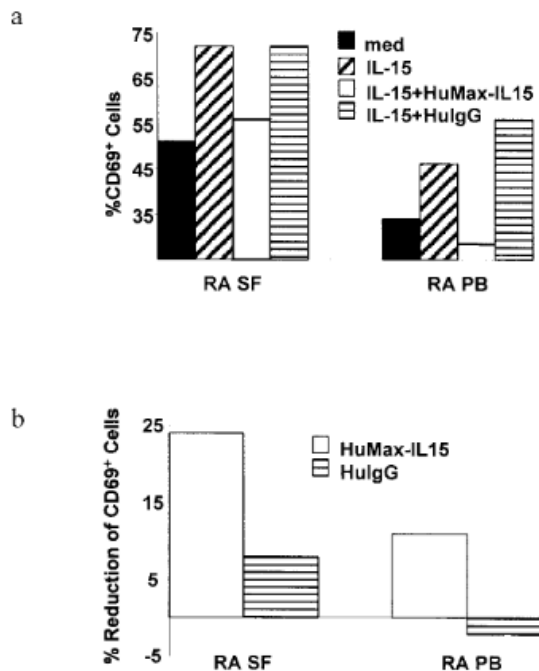


Figure 2. Effects of HuMax-IL15 (10 μ g/ml) on IL-15-dependent CD69 expression. **a**, RA peripheral blood (PB) mononuclear cells or synovial fluid (SF) mononuclear cells were rested overnight, then incubated for 24 hours with or without IL-15 (10 ng/ml) in the presence or absence of HuMax-IL15 F(ab')₂, prior to fluorescence-activated cell sorting for CD69 expression (percentage of CD69+ cells within the lymphocyte region). Data are representative of 6 similar experiments. **b**, In parallel experiments to determine the activity of HuMax-IL15 without addition of exogenous IL-15, the percentage reduction of CD69+ lymphocytes by HuMax-IL15 F(ab')₂ alone compared with huIgG F(ab')₂ was calculated. Data are representative of 6 similar experiments. See Figure 1 for other definitions.

tralized. Together these data demonstrate that HuMax-IL15 can specifically neutralize the biologic effects of IL-15 in synovial tissues *in vitro*.

Clinical effects of HuMax-IL15 in RA. Thirty RA patients, with a median age of 56 years (range 21–71 years) and a disease duration of 9.6 years (range 0.6–27 years), were randomized to initially receive a single dose of HuMax-IL15 and thereafter, assuming no dose-limiting toxicity within 4 weeks, a total of 4 infusions of HuMax-IL15 was administered at weekly intervals (Figure 3). To obtain preliminary evidence of efficacy, a pooled analysis of all dose cohorts was performed. ACR20 response (from week 0) was achieved by week 8 in 15 of 24 patients (63%), ACR50 in 9 patients (38%), and ACR70 in 6 patients (25%) (Table 1).

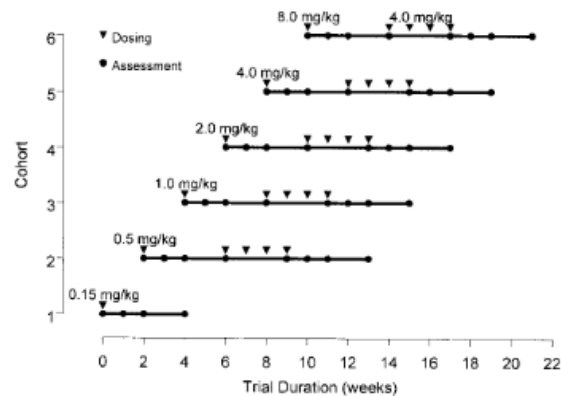


Figure 3. Clinical trial design. Patients in 6 dose cohorts received a single subcutaneous dose of HuMax-IL15 or placebo, then were observed for 4 weeks for dose-limiting toxicity. If no dose-limiting toxicity events arose, patients received weekly doses of subcutaneous HuMax-IL15 for 4 weeks under open-label conditions. The dose was increased as shown.

HuMax-IL15 tolerability in clinical study. Patients who completed and those who withdrew from the study are documented in Figure 4. Single doses of HuMax-IL15 were generally well tolerated, with no evidence of dose-limiting toxicity. There was 1 serious adverse event, a flare of RA in a patient receiving 0.15 mg/kg HuMax-IL15. However, this was not considered to be related to HuMax-IL15.

Following a single dose ($n = 30$ patients), 3 patients (1 each in the 1 mg/kg, 4 mg/kg, and 8 mg/kg cohorts) experienced 6 related adverse events, including influenza-like symptoms, transient pyrexia, and myalgia, all of which resolved spontaneously. In the multiple-dose phase ($n = 24$ patients), 9 patients reported a total of 17 related adverse events. Minor transient injection site reactions were observed in 4 patients from the group

Table 1. Clinical responses to anti-IL-15 antibody in RA patients*

Dose, mg/kg†	No. of patients	ACR responder status		
		ACR20	ACR50	ACR70
0.5	4	2	1	1
1	5	3	1	1
2	5	3	3	2
4	5	4	1	1
8-8.4	5	3	3	1
Total, no. (%)	24	15 (63)	9 (38)	6 (25)

* Anti-IL-15 = anti-interleukin-15; RA = rheumatoid arthritis; ACR20 = 20% improvement in disease activity according to the American College of Rheumatology criteria.

† Dose received in the open-label phase of the study.

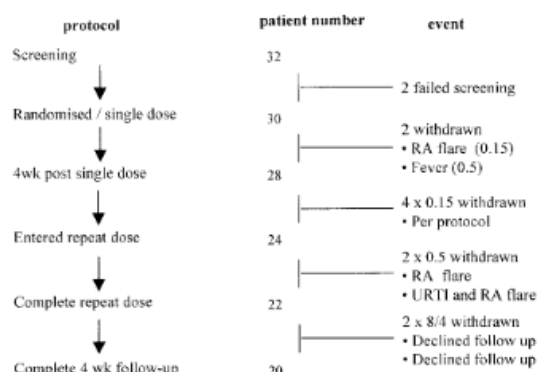


Figure 4. Outcomes through study completion in all patients screened for study enrollment. All patients are accounted for, and reasons for withdrawal are indicated. RA = rheumatoid arthritis; URTI = upper respiratory tract infection.

that received an initial dose of 8 mg/kg and subsequent doses of 4 mg/kg. Isolated events (1 patient each) that occurred in the 1 mg/kg, 2 mg/kg, and 4 mg/kg cohorts included transient pyrexia, upper respiratory tract infection (managed with oral antibiotics), herpes simplex virus, aphthous stomatitis, and influenza-like symptoms, none of which was judged to be moderate or severe. These events are consistent with those reported in similar cohorts of RA patients in other studies of biologic agents. Standard laboratory hematology and chemistry parameters were unchanged (data not shown). Results of fluorescence-activated cell sorting for leukocyte subsets, particularly T cell (CD3+, CD4+ and CD3+, CD8+) and NK cell (CD3-, CD56+) subsets, did not change from baseline up to 28 days posttreatment (data not shown). No evidence of antibodies to HuMax-IL15 was detected.

DISCUSSION

The advent of TNF-blocking therapies has brought remarkable improvement in the treatment of RA. However, partial or non-clinical responses remain a significant issue, prompting intensive exploration of the properties and therapeutic possibilities of a range of inflammatory mediators and cytokines in RA synovium. In previous studies, we have detected IL-15 in RA synovial tissues and have shown by a variety of approaches in vitro and in vivo that it could mediate proinflammatory effects therein. The present study represents a proof-of-concept translational approach to test

the hypothesis that IL-15 is proinflammatory in RA and as such provides a tractable target in synovitis.

The first series of in vitro experiments reported here showed that HuMax-IL15 can specifically neutralize biologic effects of exogenous IL-15 in RA synovial tissue. First, F(ab')₂ fragments of HuMax-IL15 suppressed proliferation of an IL-15-dependent T cell line and induced apoptosis, which signified neutralization of IL-15-mediated rescue in vitro, analogous to the neutralizing activity of human sIL-15R α (7,25). Second, IFN γ released by SFMC cultures in response to exogenous IL-15 and IL-12 was neutralized by HuMax-IL15 F(ab')₂ fragments. Third, HuMax-IL15 F(ab')₂ suppressed exogenous IL-15-induced CD69 expression in RA PBMCs and SFMCs. In addition, we found evidence that endogenous IL-15 activity was neutralized in vitro by HuMax-IL15. Thus, CD3+ T cell subsets expressing CD69 a priori were significantly reduced by HuMax-IL15 if exogenous IL-15 was not added. CD69 expression is a marker of T cell activation, but has also been implicated in T cell-macrophage interactions at a functional level (15). These data therefore suggest that IL-15 does indeed play a role in maintaining the T cell activation state in synovium. The partial inhibition observed, however, also indicates that such involvement is likely only part of a multiplicity of factors.

This is the first clinical trial to directly target IL-15 in humans. No significant toxicity emerged, although longer exposure to HuMax-IL15 will be needed to further evaluate toxicity, with an emphasis on NK and CD8 T cell memory maintenance and effector function. Evidence of a role for IL-15 in the latter arises primarily from studies of murine inflammation models and IL-15/IL-15R α gene-targeted mice (9–12). Future studies with HuMax-IL15 should provide an ideal opportunity to explore such functions of IL-15 in humans.

Although this was primarily a tolerability study, we observed encouraging evidence of efficacy. ACR response rates were similar to those observed at 4 weeks following TNF α blockade without concomitant DMARD administration (2). The absence of placebo in the open-label extension phase is an important proviso to interpretation of our data, although responses appear favorable compared with those consistently observed in placebo arms of recent trials of biologic agents in RA. Further appropriately controlled and powered studies are now required to test the efficacy of HuMax-IL15 alone and in combination with methotrexate in RA.

There remains considerable unmet clinical need in RA and in autoimmune disease despite the success of TNF α -targeting therapies. In particular, a critical objec-

tive is to achieve responses in patients in whom TNF α blockade is ineffective and to optimize responses in those exhibiting only partial benefits. Attempts at synergistic therapeutics with existing cytokine-targeting agents such as etanercept and anakinra have been disappointing (31). IL-15 operates via a signaling pathway that is expressed and functional within the synovial environment. This pathway is distinct from those signal pathways used by TNF α , IL-6, and IL-1. As such, IL-15 offers intriguing potential not only as a therapeutic target in its own right, but also as a candidate for synergistic targeting alongside TNF α .

In summary, using RA as an example, we now provide proof of concept for IL-15 as a novel candidate therapeutic target. IL-15 expression is reported in a range of autoimmune inflammatory conditions, including Crohn's disease, hepatitis, psoriasis, and psoriatic arthritis. As such, our data likely carry significance in the wider context of inflammatory diseases, although this will require confirmation in appropriately designed clinical trials.

REFERENCES

- Felson DT, Anderson JJ, Boers M, Bombardier C, Furst D, Goldsmith C, et al. American College of Rheumatology preliminary definition of improvement in rheumatoid arthritis. *Arthritis Rheum* 1995;38:727-35.
- Maini RN, Taylor PC. Anti-cytokine therapy for rheumatoid arthritis [review]. *Annu Rev Med* 2000;51:207-29.
- Lipsky PE, van der Heijde DM, St Clair EW, Furst DE, Breedveld FC, Kalden JR, et al, for the 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;343:1594-602.
- Bathon JM, Martin RW, Fleischmann RM, Tesser JR, Schiff MH, Keystone EC, et al. A comparison of etanercept and methotrexate in patients with early rheumatoid arthritis [published errata in *N Engl J Med* 2001;344:240; *N Engl J Med* 2001;344:76]. *N Engl J Med* 2000;343:1586-93.
- Grabstein KH, Eisenman J, Shanebeck K, Rauch C, Srinivasan S, Fung V, et al. Cloning of a T cell growth factor that interacts with the β chain of the interleukin-2 receptor. *Science* 1994;264:965-8.
- Bamford RN, Grant AJ, Burton JD, Peters C, Kurys G, Goldman CK, et al. The interleukin (IL) 2 receptor β chain is shared by IL-2 and a cytokine, provisionally designated IL-T, that stimulates T-cell proliferation and the induction of lymphokine-activated killer cells. *Proc Natl Acad Sci U S A* 1994;91:4940-4.
- Giri JG, Kumaki S, Ahdieh M, Friend DJ, Loomis A, Shanebeck K, et al. Identification and cloning of a novel IL-15 binding protein that is structurally related to the α chain of the IL-2 receptor. *EMBO J* 1995;14:3654-63.
- Dubois S, Mariner J, Waldmann TA, Tagaya Y. IL-15R α recycles and presents IL-15 in trans to neighboring cells. *Immunity* 2002;17:537-47.
- Waldmann TA, Tagaya Y. The multifaceted regulation of interleukin-15 expression and the role of this cytokine in NK cell differentiation and host response to intracellular pathogens [review]. *Annu Rev Immunol* 1999;17:19-49.
- Zhang X, Sun S, Hwang I, Tough DF, Sprent J. Potent and selective stimulation of memory-phenotype CD8 $^{+}$ cells in vivo by IL-15. *Immunity* 1998;8:591-9.
- Lodolce JP, Boone DL, Chai S, Swain RE, Dassopoulos T, Tretton S, et al. IL-15 receptor maintains lymphoid homeostasis by supporting lymphocyte homing and proliferation. *Immunity* 1998;9:669-76.
- Fehniger TA, Suzuki K, Ponnappan A, van Deusen JB, Cooper MA, Florea SM, et al. Fatal leukemia in interleukin 15 transgenic mice follows early expansions in natural killer and memory phenotype CD8 $^{+}$ T cells. *J Exp Med* 2001;193:219-31.
- Kennedy MK, Glaccum M, Brown SN, Butz EA, Viney JL, Embers M, et al. Reversible defects in natural killer and memory CD8 T cell lineages in interleukin 15-deficient mice. *J Exp Med* 2000;191:771-80.
- McInnes IB, al-Mughaies J, Field M, Leung BP, Huang FP, Dixon R, et al. The role of interleukin-15 in T-cell migration and activation in rheumatoid arthritis. *Nat Med* 1996;2:175-82.
- McInnes IB, Leung BP, Sturrock RD, Field M, Liew FY. Interleukin-15 mediates T cell-dependent regulation of tumor necrosis factor- α production in rheumatoid arthritis. *Nat Med* 1997;3:189-95.
- Kotake S, Schumacher HR Jr, Yarbboro CH, Arayssi TK, Pando JA, Kanik KS, et al. In vivo gene expression of type 1 and type 2 cytokines in synovial tissues from patients in early stages of rheumatoid, reactive, and undifferentiated arthritis. *Proc Assoc Am Physicians* 1997;109:286-301.
- Thurkow EW, van der Heijden IM, Breedveld FC, Smeets TJ, Daha MR, Kluin PM, et al. Increased expression of IL-15 in the synovium of patients with rheumatoid arthritis compared with patients with Yersinia-induced arthritis and osteoarthritis. *J Pathol* 1997;181:444-50.
- Oppenheimer-Marks N, Brezinschek RI, Mohamadadeh M, Vita R, Lipsky PE. Interleukin 15 is produced by endothelial cells and increases the transendothelial migration of T cells in vitro and in the SCID mouse-human rheumatoid arthritis model in vivo. *J Clin Invest* 1998;101:1261-72.
- Harada S, Yamamura M, Okamoto H, Morita Y, Kawashima M, Aita T, et al. Production of interleukin-7 and interleukin-15 by fibroblast-like synoviocytes from patients with rheumatoid arthritis. *Arthritis Rheum* 1999;42:1508-16.
- Gonzalez-Alvaro I, Ortiz AM, Garcia-Vicuna R, Balsa A, Pascual-Salcedo D, Laffon A. Increased serum levels of interleukin-15 in rheumatoid arthritis with long-term disease. *Clin Exp Rheumatol* 2003;21:639-42.
- Ziolkowska M, Koc A, Luszczkiewicz G, Ksiezopolska-Pietrzak K, Klimczak E, Chwalinska-Sadowska H, et al. High levels of IL-17 in rheumatoid arthritis patients: IL-15 triggers in vitro IL-17 production via cyclosporin A-sensitive mechanism. *J Immunol* 2000;164:2832-8.
- Cho ML, Yoon CH, Hwang SY, Park MK, Min SY, Lee SH, et al. Effector function of type II collagen-stimulated T cells from rheumatoid arthritis patients: cross-talk between T cells and synovial fibroblasts. *Arthritis Rheum* 2004;50:776-84.
- Brennan FM, Hayes AL, Ciesielski CJ, Green P, Foxwell BM, Feldmann M. Evidence that rheumatoid arthritis synovial T cells are similar to cytokine-activated T cells: involvement of phosphatidylinositol 3-kinase and nuclear factor κ B pathways in tumor necrosis factor α production in rheumatoid arthritis. *Arthritis Rheum* 2002;46:31-41.
- Groh V, Bruhl A, El-Gabalawy H, Nelson JL, Spies T. Stimulation of T cell autoreactivity by anomalous expression of NKG2D and its MIC ligands in rheumatoid arthritis. *Proc Natl Acad Sci U S A* 2003;100:9452-7.

25. Ruchatz H, Leung BP, Wei XQ, McInnes IB, Liew FY. Soluble IL-15 receptor α -chain administration prevents murine collagen-induced arthritis: a role for IL-15 in development of antigen-induced immunopathology. *J Immunol* 1998;160:5654–60.
26. Fishwild DM, O'Donnell SL, Bengoechea T, Hudson DV, Harding F, Bernhard SL, et al. High-avidity human IgG κ monoclonal antibodies from a novel strain of minilocus transgenic mice. *Nat Biotechnol* 1996;14:845–51.
27. Villadsen LS, Schuurman J, Beurskens F, Dam TN, Dagnaes-Hansen F, Skov L, et al. Resolution of psoriasis upon blockade of IL-15 biological activity in a xenograft mouse model. *J Clin Invest* 2003;112:1571–80.
28. Gracie JA, Forsey RJ, Chan WL, Gilmour A, Leung BP, Greer MR, et al. A proinflammatory role for IL-18 in rheumatoid arthritis. *J Clin Invest* 1999;104:1393–401.
29. Fries JF, Spitz PW, Kraines RG, Holman HR. Measurement of patient outcome in arthritis. *Arthritis Rheum* 1980;23:137–45.
30. Ortiz AM, Laffon A, Gonzalez-Alvaro I. CD69 expression on lymphocytes and interleukin-15 levels in synovial fluids from different inflammatory arthropathies. *Rheumatol Int* 2002;21:182–8.
31. Genovese MC, Cohen S, Moreland L, Lium D, Robbins S, Newmark R, et al, for the 20000223 Study Group. Combination therapy with etanercept and anakinra in the treatment of patients with rheumatoid arthritis who have been treated unsuccessfully with methotrexate. *Arthritis Rheum* 2004;50:1412–9.