NOD-scid IL2R $\gamma^{null}$ mice engrafted with human peripheral blood mononuclear cells as a model to test therapeutics targeting human signaling pathways

Maryam Zadeh-Khorasani1, Thomas Nolte2, Thomas D Mueller3, Markos Pechlivanis3, Franziska Rueff4, Andreas Wollenberg4, Gert Fricker5, Eckhard Wolf2, Matthias Siebeck1 and Roswitha Gropp1*

Abstract

Background: Animal models of human inflammatory diseases have limited predictive quality for human clinical trials for various reasons including species specific activation mechanisms and the immunological background of the animals which markedly differs from the genetically heterogeneous and often aged patient population.

Objective: Development of an animal model allowing for testing therapeutics targeting pathways involved in the development of Atopic Dermatitis (AD) with better translatability to the patient.

Methods: NOD-scid IL2R $\gamma^{null}$ mice engrafted with human peripheral blood mononuclear cells (hPBMC) derived from patients suffering from AD and healthy volunteers were treated with IL-4 and the antagonistic IL-4 variant R121/Y124D (Pitrakinra). Levels of human (h)IgE, amount of B-, T- and plasma- cells and ratio of CD4 : CD8 positive cells served as read out for induction and inhibition of cell proliferation and hIgE secretion. Results were compared to in vitro analysis.

Results: hIgE secretion was induced by IL-4 and inhibited by the IL-4 antagonist Pitrakinra in vivo when formulated with methylcellulose. B-cells proliferated in response to IL-4 in vivo; the effect was abrogated by Pitrakinra. IL-4 shifted CD4 : CD8 ratios in vitro and in vivo when hPBMC derived from healthy volunteers were used. Pitrakinra reversed the effect. Human PBMC derived from patients with AD remained inert and engrafted mice reflected the individual responses observed in vitro.

Conclusion: NOD-scid IL2R $\gamma^{null}$ mice engrafted with human PBMC reflect the immunological history of the donors and provide a complementary tool to in vitro studies. Thus, studies in this model might provide data with better translatability from bench to bedside.
Human peripheral blood mononuclear cells (hPBMC) are widely used for drug screening [5-8], but the example of TGN 1412 has demonstrated that experimental conditions might lead to difficulties in interpretation and translatability of data [9]. Immunological reactions are very often modulated and fine-tuned by temporary, spatial and cell-specific expression of the respective cytokines and their cognate receptors [10,11] and in vitro culture conditions may inadequately reflect cell–cell interactions in lymphoid organs in response to an immunological trigger [9].

Therefore, immune-compromised NOD-scid IL2Rγnull mice engrafted with human PBMC have become alternative models to study chronic inflammatory diseases such as rheumatoid arthritis [12,13], AD and ulcerative colitis (UC) [14,15]. It has been shown in the AD model, that the immunological background of the donor is crucial to the induction of atopic dermatitis like features and that the immunological imprinting is preserved in vivo [14].

AD is a T-helper cell type 2 (Th2) driven inflammation with interleukin-4 (IL-4) and interleukin 13 (IL-13) playing key roles in the early edematous phase [16-18]. IL-4 activates the type I receptor complex consisting of the IL-4 receptor α (IL-4Ra) and the so called common gamma (γc) chain which is thought to be responsible for proliferation and differentiation of T-cells. Both interleukins, IL-4 and IL-13, can also activate the type II interleukin-4 receptor complex consisting of IL-4Ra and the IL-13 receptor α1 (IL-13α1) chain resulting in the generation of phenotypic symptoms such as the immunoglobulin class switch from IgM to IgG4 and IgE, fibrosis, epithelial hyperplasia and barrier dysfunction [19-21]. Therefore, both IL-4 receptor complexes have been identified as therapeutic targets. One promising approach is the development of the antagonistic IL-4 variant R121D/Y124D (Pitrakinra), which inhibits the formation and activation of the IL-4 type I and II receptor complexes and has already been tested successfully in clinical phase II studies in asthma [22]. However, limited half-lives of IL-4 or mutant proteins are still a major hurdle in their therapeutic use which results in the need for daily applications of high doses [22-24].

Here we show that studies in NOD-scid IL2Rγnull mice engrafted with PBMC complement in vitro studies with cultured hPBMC. IL-4 induced the secretion of IgE in vitro and in vivo and Pitrakinra abolished the effect of IL-4 as expected. In contrast to previous studies, formulation with methylcellulose was instrumental to the activating and inhibitory effect of IL-4 and Pitrakinra, respectively. The proliferation-inducing effect was not reflected in the spleen of engrafted mice; however, differentiation of T-cells was similar in vitro and in vivo. Cell batches which responded poorly in vitro were inert to IL-4 treatment after transplantation into NOD-scid IL2Rγnull mice in vivo, indicating that the immunological characteristics of the human donors are preserved in the animal hosts and that results of studies in this humized model might be better translatable to the patient.

Material and methods

Production of wild-type IL-4 and Pitrakinra

Wildtype human IL-4 as well as the IL-4 antagonist/inhibitor Pitrakinra were produced in E. coli as described [25]. The amino acid exchanges R121D/Y124D in IL-4 leading to the IL-4 inhibitor known as Pitrakinra were introduced by a two-step PCR mutagenesis and correctness of the cDNA was verified by dideoxy sequencing. For protein production, the cDNA encoding for the mature part of human IL-4 or the variant R121D/Y124D was cloned into the E. coli expression vector RBSIP5205X/o [26]. Transformed E. coli cells of the strain BL21 (DE3) were grown in LB medium until an optical density of 0.6 to 0.8 at 600 nm was reached. Protein expression was induced by addition of 1 mM IPTG (isopropyl-β-thiogalactosid), expression was continued for a further three hours at 37°C. Cells were harvested by centrifugation and lysed by ultrasonication. IL-4 as well as the variant Pitrakinra were expressed in insoluble form as inclusion bodies, which were dissolved in 20 volumes (v/w) of 6 M guanidinium hydrochloride (GuHCl), 50 mM Tris–HCl pH 8.0. The denatured protein was refolded by a two-step protocol, the first step comprising of a rapid five-fold dilution of the protein solution (protein concentration 2 mg/ml) in 6 M GuHCl into ice-cold water. The solution was stirred for 15 min and then dialyzed against 20 volumes phosphate buffered saline (20 mM sodium phosphate, 120 mM NaCl, 2 mM KCl, pH 7.4) for 24 h at 4°C. The protein solution was buffered to pH 5.5 using 4 M ammonium acetate pH 4.5. Insoluble protein precipitate was removed by centrifugation and the clear supernatant was loaded onto a cation exchange column (GE Healthcare CM sepharose FF). IL-4 was eluted by applying a linear gradient of 0 to 1 M sodium chloride in 20 mM ammonium acetate pH 5.5. IL-4 containing fractions were pooled and subjected to a second purification employing a reversed phase HPLC chromatography. Highly pure IL-4 protein was eluted using a linear gradient of 0.1% trifluoroacetic acid to 100% acetonitrile. Purity was checked by SDS-PAGE and ESI-FT-ICR mass spectrometry. As a quality control, the biological activities and receptor binding properties were examined by measuring the dose dependency of TF-1 cell proliferation for wild-type human IL-4 (EC50 of about 10 to 20 pM) as described, or for Pitrakinra by dose-dependent inhibition of the TF-1 cell proliferation [27]. Increasing concentrations of Pitrakinra were added to TF-1 cells, which were simultaneously stimulated with 100 pM wild-type IL-4, the half-maximal concentration of inhibition of TF-1 cell proliferation IC50
was determined to be about 5 nM. In addition binding affinity of the recombinant IL-4 proteins (wild-type and the variant Pitrakinra) to its high-affinity receptor IL-4Rα was determined by surface plasmon resonance was described previously [28].

Isolation and engraftment of human PBMC

Peripheral blood was collected from patients suffering from AD and healthy volunteers. Human PBMC were purified by Ficoll-paque density gradient centrifugation. 30 ml of blood in sodium citrate solution was diluted with 30 ml of HANKS Balanced Salt Solution (Sigma Aldrich, Deisenhofen, Germany) and loaded on leuko-sep Tubes (Greiner Bio One, Frickenhausen, Germany). Cells were separated at 800 x g for 15 min at RT according to the manufacturer’s instruction. Human PBMC were isolated, washed with HANKS Balanced Salt Solution supplemented with 2500 IE Heparin Sodium (Braun) and resuspended in phosphate buffered saline (PBS) at a density of 20 x 10⁶/ml.

Six to 16 weeks old NOD-scid IL2Rγnull mice were engrafted with 200 μl of the cell suspension (4 x 10⁶ cells) by intravenous injection. The average weight 24.5 ± 4 g. Animals were randomized for age and sex. Following engraftment the animals rested for 7 days.

Cell culture

4x10⁶ hPBMC were resuspended in 2 ml RPMI, 10% FCS (v/v), 1 mM sodium pyruvate, 10 U Penicillin/Streptomycin, 2 mM Glutamine (Sigma, Deisenhofen, Germany) and loaded on Leuco-sep Tubes (Greiner Bio One, Frickenhausen, Germany). Cells were separated at 800 x g for 15 min at RT according to the manufacturer’s instruction. Human PBMC were isolated, washed with HANKS Balanced Salt Solution supplemented with 2500 IE Heparin Sodium (Braun) and resuspended in phosphate buffered saline (PBS) at a density of 20 x 10⁶/ml.

Study protocol

NOD.cg-Prkdcscid Il2rgtm1Wjl/Szj (abbreviated as NOD-scid IL2Rγnull) mice were obtained from Charles River Laboratories (Sulzfeld, Germany). The mice were housed under specific pathogen free (SPF) conditions in individually ventilated cages. The facility is controlled under specific pathogen free (SPF) conditions in individual ventilated cages. The facility is controlled according to FELASA guidelines. Methylcellulose (A4C Prem) was purchased from Fagron GmbH, Barsbüttel, Germany.

Mice were engrafted on day one. Seven days post engraftment NOD-scid IL2Rγnull mice (6–16 weeks old) were treated for five consecutive days (day 8–12) intraperitoneally with 10 μg IL-4 dissolved in PBS, or 10 μg IL-4 dissolved in 0.5% methylcellulose (w/v), 0.05% TWEEN 80 (v/v) in PBS or 10 μg IL-4 plus 100 μg Pitrakinra dissolved in 0.5% methylcellulose (w/v), 0.05% (v/v) TWEEN 80 in PBS.

Mice were inspected daily and sacrificed at day 30.

Serum hIgG and hIgE levels

Samples were blinded and human serum IgG levels were measured turbidimetrically using COBAS INTEGRA 800 (Roche, Penzberg, Germany). Human serum IgE levels were measured by the Elecsys 2010 Immuno-assay (Roche, Penzberg, Germany). Murine IgE levels were analyzed by Mouse IgE ELISA kit according to the manufacturer’s instruction (BD Biosciences, Heidelberg, Germany).

Flow cytometry analysis

Surface phenotyping of human lymphocytes

Labeling of the human lymphocytes, in human and mouse, was performed using the following monoclonal antibodies (mAbs): Anti-human CD3- FITC (clone SK7), anti-human CD4-PE (clone SK3), anti-human CD8-APC (clone SK1), anti-human CD19-APC (clone SJ25C1), anti-human CD38-PE (clone HIT2), anti-human CD45-APC-H7 (clone 2D1), anti-human CD56-PerCP-CY™ 5.5 (clone B159), anti-human CD138-FITC (clone M115). Anti-mouse CD45 –PE-Cy™ 7 (clone30-F11) staining was performed to exclude all murine host cells from the analysis as well as to check for cross-reactivity with human cells. All mAbs were purchased from BD Biosciences, Heidelberg, Germany.

Human peripheral blood was taken from the vein in heparinized tubes. At the time of sacrifice peripheral mouse blood was collected in heparin coated tubes from the anesthetized mouse by cardiocentesis. 100 μl of blood was stained with appropriate antibodies and incubated for 15 min at room temperature in darkness. The erythrocytes were removed from the samples using 1× BD FACs Lysing Solution (BD Biosciences, Heidelberg, Germany) for 10 min at RT and darkness.

Single cell suspensions were prepared from the spleen in RPMI-1640 containing 10% FCS by mincing with a metal mesh followed by a 100 μm cell strainer (BD Biosciences). The cells were treated by 1× BD Pharm lyse (BD Biosciences, Heidelberg, Germany) for 10 min at RT. Following the washing process in 2% RPMI-1640 (Sigma-Aldrich, Germany), 10⁶ cells/ml were labeled with appropriate antibodies.

Following staining the cells were washed in FACS buffer (PBS containing 2% FCS) and resuspended in 500 μl of the same.

At least 10,000 events were measured using FACS Canto (BD Biosciences, Heidelberg, Germany). Post-acquisition
data were analyzed using the FlowJo 7.6.5 software (Tree Star, Ashland, OR).

Statistical analysis
Statistical analyses were performed using R a free software environment for statistical computing and graphing (www.r-project.org). Group means were compared with analysis of variance (ANOVA), followed by Tukey’s test for multiple comparisons. Where assumptions for ANOVA were not fulfilled, Kruskal Wallis test was applied.

Ethical considerations
All donors gave informed written consent and the study was approved by the IRB of the Medical Faculty at the University of Munich.

All animal studies were approved by the Ethics Committee for animal use of the government of Upper Bavaria, Germany (55.2-1-54-2531-57-10) and performed in compliance with German Animal Welfare Laws.

Results
Formulation of IL-4 with methylcellulose induces the secretion of hlgE
In order to examine whether the formulation of IL-4 with methylcellulose enhances the secretion of hlgE in NOD-scid IL2Rγnull mice engrafted with hPBMC, hlgE levels were measured in animals treated with 10 μg IL-4 in PBS (IL-4, n = 10) and animals treated with 10 μg IL-4 formulated with 0.5% methylcellulose, 0.05% TWEEN 80 in PBS (IL-4 + Methylcellulose, n = 12). Engrafted animals treated with carrier alone (Methylcellulose, n = 4) served as control. As we knew from previous experiments [14,15], that the responsiveness to IL-4 is dependent on the immunological background of the donors and their current immunological status, all hPBMC were analyzed simultaneously in vitro with respect to their capacity to secrete IgE in response to IL-4. Human PBMC were isolated as described in material and methods and 4 × 10^6 cells were incubated for 14 days with 50 ng/ml IL-4 as previously described [29]. The induction of IgE synthesis was measured in the supernatant by immuno assay and turbidimetric measurement. Human PBMC from two volunteers without any symptoms of AD and thus considered as ‘healthy’ responded similarly to IL-4 in vitro. Human PBMC from one donor (non-AD1) who had no history of allergy displayed mean values of IgE of 200 ± 94 ng/ml (n = 6) in vitro, whereas hPBMC from the other donor (non-AD2) who experienced rare allergic reactions displayed mean IgE values of 275 ± 126 ng/ml (n = 4). Human PBMC of these donors were repeatedly isolated and engrafted into NOD-scid IL2Rγnull mice; cohorts consisted of four to six animals. In addition, two donors suffering from AD (AD1, AD2) were included in the study. The isolated hPBMC of both responded upon exposure to IL-4 in vitro with the secretion of hlgE (244 and 4248 ng/ml, respectively). Eight days post engraftment mice were treated for five consecutive days as described above according to the treatment regime defined by the respective regime of the group. At day 30 all animals were sacrificed and their blood was analyzed for hlgE levels. As shown in Figure 1 we observed elevated levels only when IL-4 was formulated with methylcellulose. In this group the median level was 33.1 ng/ml as compared to 1.8 ng/ml in the group treated with IL-4 dissolved in PBS and 0 ng/ml in control group treated with carrier alone. The analysis of the data revealed that hlgE levels were significantly different (Kruskal-Wallis, p = 0.013). In this experiment, we observed no difference between the AD and non-AD groups.

The formulation with methylcellulose prolongs the presence of IL-4 in vivo
In order to examine whether the formulation with methylcellulose results in a prolongation of the half-life of IL-4 in vivo, hIL-4 levels were measured in mouse sera as described in material and methods. Blood was drawn at day 9, 24 h after hIL-4 administration in engrafted animals treated with IL-4 dissolved in PBS (n = 4), methylcellulose (n = 4) and IL-4 dissolved in methylcellulose (n = 11). As shown in Figure 2 hIL-4 levels in mouse sera were below detection level in the “IL-4” and “Methylcellulose” groups and reached a median of 1.95 ng/ml in the “IL-4 + Methylcellulose” group which was significantly different from the other groups. (ANOVA, F = 4.3, p = 0.03).

The inhibitory effect of the IL-4 variant Pitrakinra on hlgE secretion in vitro and in vivo
In order to examine whether this animal model might be useful to study inhibitors addressing targets of human inflammatory pathways we analyzed the effect of the IL-4 variant R121D/Y124D (henceforward the variant will be referred to as antagonist or Pitrakinra) in NOD-scid IL2Rγnull mice engrafted with hPBMC. To determine the correct dosage of the IL-4 antagonist, hPBMC derived from healthy volunteers and patients suffering from AD were incubated in vitro with 50 ng/ml IL-4 in the absence or presence of increasing concentrations of the antagonist (50–500 ng/ml). As in previous experiments hlgE levels were measured by turbidimetry at day 14 with high variability in hlgE levels being seen in both groups upon exposure to IL-4. In order to normalize the inhibitory capacity of the IL-4 antagonist, hlgE values measured in the sample exposed to IL-4 were defined as 100% and the inhibitory effect of antagonist as % change in comparison to the sample treated with IL-4 alone. As shown in Figure 3A secretion of hlgE decreased with increasing antagonist concentration, however, the susceptibility
to the antagonist seemed to be different in the AD as compared to the non-AD group. At a concentration of 100 ng/ml antagonist the median change in the AD group was 33.6%, while in the non-AD group the median was 89% and this was statistically significant (Kruskal-Wallis rank sum test, p = 0.02). When the antagonist concentration exceeded the IL-4 concentration by a factor of 10 the remaining hIgE levels were close to zero in both groups. Therefore, for in vivo studies animals were treated with 10 μg IL-4 in methylcellulose or 10 μg IL-4 plus 100 μg Pitrakinra in methylcellulose. Otherwise, treatment regimes were identical to those used in the previous experiments. Animals were engrafted with hPBMC derived from four donors suffering from AD and two without any history of atopic dermatitis. Cohorts consisted of four to six animals. As in the in vitro experiments the values were normalized. Mean values of hIgE in the IL-4 treated cohorts were defined as 100% and compared to mean values of the cohorts treated with IL-4 and antagonist. As shown in Figure 3B the median inhibition of hIgE secretion was 22%. The differences between both groups were not statistically significant, but a strong trend was indicated (p = 0.07; Kruskal-Wallis rank sum test).

FACS analysis of hPBMC and splenic human lymphocytes
In order to analyze effect of Pitrakinra on T- and B-cells in vivo and in vitro, hPBMC and isolated human lymphocytes were subjected to FACS analysis. hPBMC
isolated from different donors (AD n = 5, non-AD n = 8) were incubated with 50 ng IL-4 or 50 ng IL-4 + 500 ng antagonist as previously described. After 14 days the total numbers of cells were counted in 100 μl of the cell culture.

For the in vivo studies animals were engrafted and treated as in the previous study. The AD group consisted of three different donors and the non-AD group consisted of two different donors. The groups were further subdivided into the three different treatment groups: “No treatment”, “IL-4” and “IL-4 + Antagonist” (Table 1).

Cohorts consisted of three to six animals. At day 30 the animals were sacrificed and the splenic lymphocytes were isolated as described in material and methods. Engraftment levels were determined by counting of human CD45 positive cells among splenic lymphocytes. In both groups engraftment levels varied significantly and ranged from 0.3 to 71%. There was a strong trend that hPBMC from patients with AD did engraft at higher levels (p = 0.06; Kruskal-Wallis rank sum test). The median engraftment level in the AD group was 16% as compared to the median of 5.5% in the non-AD group. Treatment regimen had no effect on engraftment levels. Animals with engraftment levels below 1% were considered non-engrafted and excluded from the analysis. This criterion applied to six animals of the AD group and 12 of the non-AD group (Table 1).

Table 1 Number of animals in the study

| Diagnosis | AD | Non-AD |
|-----------|----|--------|
| Animals | Engrafted | At the end of experiment | Analyzed | Δ | Engrafted | At the end of experiment | analyzed | Δ |
| Treatment | | | | | | | | |
| No | 4 | 3 | 3 | 1 | 10 | 10 | 8 | 2 |
| IL-4 | 18 | 16 | 15 | 3 | 20 | 20 | 14 | 6 |
| IL-4 + Antagonist | 19 | 18 | 13 | 6 | 10 | 9 | 5 | 5 |
| Total number | 41 | 37 | 31 | 10 | 40 | 39 | 27 | 13 |
The effect of Pitrakinra on B- and plasma-cells in vitro and in vivo

As IL-4 is known to induce proliferation and differentiation of B-cells we analyzed the impact of IL-4 and antagonist in vitro and in vivo by FACS analysis of isolated hPBMC and isolated human splenic lymphocytes from the engrafted animals. For in vitro analysis, the number of B-cells (CD19⁺) was determined in 100 μl of the cell suspension by FACS analysis. For in vivo analysis, splenic B-cells (CD19⁺) were isolated from the spleen as described in material and methods and the percentage of B-cells was determined by FACS analysis. As shown in Figure 4, treatment with IL-4 resulted in a decline of B-cells after 14 d incubation without any treatment and IL-4 had only a minor effect on total B-cell numbers in vitro. In contrast, IL-4 had a pronounced effect on plasma-cells in vitro (CD19⁺ + CD138⁺). At day 1 (median = 3) almost no plasma-cells were detectable. The cell culture conditions alone seemed to induce differentiation of plasma-cells as shown by the increase of the median at day 14 (median = 148), an effect which was enhanced by IL-4 (median = 201) and abrogated by antagonist (median = 87). Statistical analysis of log transformed data revealed that the “IL-4” group was significantly different from all the other groups (ANOVA, F = 6.3, p = 0.01).

These results differ from those obtained from the analysis of human lymphocytes recovered from mouse spleens. Here, treatment with 10 μg IL-4 plus methylcellulose resulted in an increase of B-cells (median = 5.8%) as compared to the non-treated group (median = 1%) and treatment with 10 μg IL-4 plus 100 μg antagonist reduced the percentage of B-cells (median = 0.82%). Statistical analysis revealed that the “IL-4” group was significantly different from the “IL-4 + Antagonist” group (p = 0.05). In contrast, no effect on plasma cells was observed in vivo.

The effect of Pitrakinra on T-cells in vitro and in vivo

In order to analyze the effect of IL-4 and Pitrakinra on T-cells and subsets of T-cells, human T-cells from the in vitro and in vivo experiments were subjected to FACS analysis. As shown in Figure 5, IL-4 had a strong...
proliferation inducing effect on T-cells in vitro. The effect was more pronounced in the non-AD group (n = 4), where total numbers increased by a factor of 3.9 (median = 51078) as compared to a factor of 2.8 in the AD-group (n = 7; median = 48040). The difference was not statistically significant. IL-4 and Pitrakinra had no effect on proliferation of T-cells in the spleen of the engrafted mice (data not shown).

As IL-4 is known to influence the ratio of CD4\(^+\) : CD8\(^+\) T-cells we further analyzed the ratios in vitro and in vivo. Although IL-4 induced the proliferation of both, CD4\(^+\) and CD8\(^+\) cells (data not shown), the effect was more pronounced in CD4\(^+\) cells. Unexpectedly, this effect was only observed in the non-AD group. As shown in Figure 5 the ratio in the AD group did not change significantly and was not affected by the antagonist. Mean values were 2.4 ± 0.8 at day 1, slightly increased during the incubation period to 3.6 ± 1.95 at day 14 in the absence of IL-4 and to 4.2 ± 1.16 in the presence of IL-4 and remained at a level of 4.1 ± 0.75 in the presence of antagonist. This was profoundly different in the non-AD group where mean values increased from 2.2 ± 0.6 at day 1 to 3.9 ± 7.2 at day 14 in the absence of IL-4 and significantly increased by a factor of 1.8 to a level of

![Figure 5](http://www.translational-medicine.com/content/11/1/4)
7.2 ± 1.7. Pitrakinra partially reversed the effect of IL-4 and caused a decrease to a level of 5 ± 2. Statistical analysis revealed that the “IL-4” group was statistically different from all other groups (F = 6.5, p = 0.008 ANOVA multiple comparison).

To test whether these responses were reflected in engraved animals, we compared the CD4+ : CD8+ ratios in response to IL-4 and antagonist of individual donors in vitro and in vivo. The analysis of CD4+ : CD8+ ratios revealed that the pattern was preserved (Figure 5) whereby the non-AD donor was markedly stimulated by IL-4 (No treatment, n = 6; IL-4, n = 11, IL-4 + Antagonist, n = 5), AD1 moderately stimulated (No treatment, n = 3; IL-4, n = 4, IL4 + Antagonist, n = 4) and AD2 not stimulated (IL-4, n = 7; IL4 + Antagonist, n = 5) by IL-4 and antagonist in vitro and in vivo.

Discussion
In this study we describe the development of an animal model that closely reflects the immunological background of the individual human donor and allows for the studying of inhibitors addressing human therapeutic targets involved in chronic inflammatory diseases such as AD in vivo. NOD-scid IL2Rγnull mice were engraved with hPBMC derived from patients suffering from AD and from healthy volunteers and treated with IL-4 or the IL-4 antagonist/inhibitor Pitrakinra. We chose IL-4 and its inhibitor Pitrakinra as a model system as the effects of both have been thoroughly characterized in vitro and both have been examined in clinical studies [22,24]. A major problem with in vivo studies has been the short half-life of IL-4 and Pitrakinra [31,32]. In contrast to previous studies [30,32] we observed no effect when IL-4 was dissolved in PBS which may reflect that in our experiments approximately a tenth of the cell numbers of hPBMC were used for engraftment or the use of different mouse strains. In our studies, hIgE secretion was significantly induced when IL-4 was formulated with methylcellulose, irrespective of the atopic background of the donor. Finally, the IgE secretion reflected the observed induction in vitro. The effect was most probably due to an inhibition of the rapid secretion of IL-4 by methylcellulose. In contrast to IL-4 administered with PBS, IL-4 formulated with methylcellulose could be detected in the sera of respective mice 24 h post administration in therapeutic concentrations. However, we cannot conclude from these studies, that long time exposure to IL-4 is also required for priming dendritic cells or T-cells since Biedermann et al. [33] have shown that short time exposure to IL-4 without formulation is sufficient to prime dendritic cells in vivo.

Next we analyzed whether the inhibitory effect of Pitrakinra on IgE secretion is reflected in this model. Surprisingly hPBMC derived from AD patients and healthy volunteers responded differently to Pitrakinra in vitro. At lower concentration there seemed to be no effect on hIgE secretion in the AD group, while the non-AD group responded to the IL-4 inhibitor. At a ratio of 1: 10 of IL-4 and antagonist both groups responded similarly and the inhibition was also seen in the engraved animals.

The effect of IL-4 and Pitrakinra on B- and plasma cells produced mirror image results when in vitro and in vivo experiments were compared, indicating that in this case the environment of the spleen may influence the action and effect of immune-modulatory agents. In the spleen IL-4 induced the proliferation of B-cells and Pitrakinra reversed this effect, while in vitro B-cell numbers did not increase. Conversely, plasma cells proliferated in vitro in response to IL-4 and Pitrakinra whereas in the spleen no effect of IL-4 and Pitrakinra was detected.

Opposing effects of IL-4 on T-cells were observed in vitro and in vivo studies. As expected, IL-4 induced significant proliferation of T-cells in vitro, an effect that was significantly inhibited by Pitrakinra. In contrast, treatment with IL-4 or Pitrakinra had no effect on engraftment- or T-cell levels in in vivo studies.

As expected, IL-4 exerted its capacity to differentiate T-cells in vitro and in vivo similarly. IL-4 tipped the balance of CD4+ : CD8+ in favor of CD4+ cells an effect that was abrogated by Pitrakinra. An interesting and unexpected observation in these experiments was the apparent influence of the immunological background of the donor on the response. PBMC from AD patients hardly responded in in vitro studies and individual responses seen in vitro were well reflected in vivo studies.

Recent studies have shown that high density cell culture of hPBMC mimics cell-cell interactions in lymphoid organs [9]. In our experiments, exposure to IL-4 for 24 h did not prime the cells to produce IgE (data not shown); instead long time incubation was required. The observed individual responses to IL-4 and Pitrakinra indicate that analysis of hPBMC may provide evidence for potential responses in clinical trials. The analysis of the same hPBMC in engrafted mice further allows for detailed studies in lymphoid organs.

In summary, we present an animal model which has the potential to provide data with higher translatability to the patient as compared to conventional mouse models. It complements in vitro studies and can be used to stratify patients for later clinical studies.

Competing interest
The authors declare that they have no competing interests.

Authors’ contributions
M2K carried out animal studies and FACS analysis. TN carried out animal studies and participated in coordination. TM and MP synthesized IL-4 and Pitrakinra. FR was responsible for patient recruitment and anamnesis. AW participated in FACS analysis. GF formulated the IL-4 and Pitrakinra. EW
conceived of the study and participated in writing the manuscript. MS participated in design of the study and the statistical analysis. RG designed the study, analyzed the data and wrote the manuscript. All authors read and approved the final manuscript.

Acknowledgements
This work was funded by the Bundesministerium für Bildung und Forschung (grant number PTI 0315466). The authors thank Alice Schaaf for excellent technical assistance. We thank Origenis GmbH for generous support.

Author details
1 Department of Surgery, Klinikum der Ludwig-Maximilians Universität München, Munich 80336, Germany. 2 Institute of Molecular Animal Breeding and Biotechnology, and Laboratory for Functional Genome Analysis (LAFUGA), Gnee Center, LMU Munich, Munich 81377, Germany. 3 Julius von Sachs Institute, University of Würzburg, Würzburg 87082, Germany.

Received: 19 October 2012 Accepted: 25 December 2012
Published: 7 January 2013

Reference
1. Suntharalingam G, Perry MR, Ward S, Brett SJ, Castello-Cortes A, Brunner MD, et al: Cytokine storm in a phase 1 trial of the anti-CD28 monoclonal antibody TGN1412. N Engl J Med 2006, 355:1018–1028.
2. Keller F, Furr U, Niewenhuizen EE, Blumgang BS, Strobl W: Oxazolone colitis, a Th2 colitis model resembling ulcerative colitis, is mediated by IL-13-producing NK-T cells. Immunity 2002, 17:629–638.
3. Man MQ, Hatano Y, Lee SH, Man M, Chang S, Feingold KR, et al: Characterization of a hapten-induced, murine model with multiple features of atopic dermatitis: structural, immunological, and biochemical changes following single versus multiple oxazolone challenges. J Invest Dermatol 2006, 128:70–76.
4. Daniel C, Sartory NA, Zahn N, Schmidt R, Geisslinger G, Radeke HH, et al: FT270 ameliorates oxazolone colitis in mice by directly affecting T helper type 2 functions. Mol Immunol 2007, 44:3305–3316.
5. Berk L, Mitra MM, Kreitsberg J, Bedrosian CL, Tolcher AW, Clackson T, et al: Analysis of the pharmacodynamic activity of the mTOR inhibitor ridaforolimus (AP23573, MK-8669) in a phase 1 clinical trial. Cancer Chemother Pharmacol 2012, 69:1369–1377.
6. Dhir V, Fort M, Mahmood R, Higbee R, Warren W, Narayanan P, et al: A predictive biomimetic model of cytokine release induced by TGN1412 and other therapeutic monoclonal antibodies. J Investigative Dermatology 2012; 95:4–42.
7. Ji J, Kendis RJ, Zhang Y, Rubinstein L, Zhang Y, Kost M, et al: Characterization of the pharmacodynamic response to the poly(ADP-Ribose) polymerase inhibitor ABT-888 in human peripheral blood mononuclear cells. PLoS One 2011, 6:e26152.
8. Comin-Andiuk B, Chodon T, Szecsi H, Matsuura D, Mock S, Jall J, et al: The oncogenic Braf kinase inhibitor PLX4032/RG7204 does not affect the viability or function of human lymphocytes across a wide range of concentrations. Clin Cancer Res 2010, 16:6040–6048.
9. Romer PS, Berr S, Avota E, Na SY, Battaglia M, ten Berge I, et al: The role of PBMC at high cell density increases sensitivity of T-cell responses, revealing cytokine release by CD28 superagonist TGN1412. Blood 2011, 118:6772–6782.
10. LaPorte SL, Juo ZS, Vaclavikova J, Colf LA, Qi X, Heller NM, et al: Inhibition of CD23 processing correlates with inhibition of IL-4-stimulated IgE production in human PBL and hu-PBL-reconstituted SCID mice. Clin Exp Allergy 2009, 39:250–259.
11. Zadeh-Khorasani A, Soma Y, Kaya W, Mizooguchi M: Flow cytometric analysis of IL-4, IL-13 and IFN-gamma expression in peripheral blood mononuclear cells and detection of circulating IL-13 in patients with atopic dermatitis provide evidence for the involvement of type 2 cytokines in the disease. J Dermatol Sci 2002, 29:19–25.
12. Wollenberg A, Kraft S, Oppel T, Bieber T: Atopic dermatitis: pathogenic mechanisms. Clin Exp Dermatol 2000, 25:530–534.
13. Elash IA, Lee CG, Zheng T, Ma B, Horner RL, Zhu Z: New insights into the pathogenesis of asthma. J Clin Invest 2003, 111:291–297.
14. Thomas Nolte MZ-K, Sartovar O, Rueff F, Varga R, Herbach N, Wanke R, Wollenberg A, Mueller T, Gropp R, Wolf E, Siebeck M: Induction of oxazolone mediated features of atopic dermatitis in NOD-scid IL2Rnull mice engrafed with human peripheral blood mononuclear cells. Dis Model Mech 2013, 6:125–134.
15. Thomas Nolte MZ-K, Sartovar O, Rueff F, Gülberg V, Herbach N, Wollenberg A, Mueller T, Siebeck M, Wolf E, Gropp R: Oxazolone and ethanol induce colitis in NOD-scid IL2Rnull guill mice engrafed with human peripheral blood mononuclear cells. Clin Exp Immunol 2013, accepted for publication.
16. Obara W, Kaya Y, Ra C, Nishioka K, Soma Y, Mizooguchi M: T cells and mast cells as a major source of interleukin-13 in atopic dermatitis. Dermatology 2002, 205:11–17.
17. Kaminishi K, Soma Y, Kaya W, Mizooguchi M: Flow cytometric analysis of IL-4, IL-13 and IFN-gamma expression in peripheral blood mononuclear cells and detection of circulating IL-13 in patients with atopic dermatitis provide evidence for the involvement of type 2 cytokines in the disease. J Dermatol Sci 2002, 29:19–25.
18. Wollenberg A, Kraft S, Oppel T, Bieber T: Atopic dermatitis: pathogenic mechanisms. Clin Exp Dermatol 2000, 25:530–534.
19. Elash IA, Lee CG, Zheng T, Ma B, Horner RL, Zhu Z: New insights into the pathogenesis of asthma. J Clin Invest 2003, 111:291–297.
20. T helper 2 function in asthma. J Dermatol Sci 2002, 29:19–25.
21. Grewe M, Yu Guo, G, Schepf F, Krumm A: Lesional expression of interferon-gamma in atopic eczema. Lancet 1994, 343:25–26.
22. Wenzel S, Willibrand DH, Fuller R, Getz EB, Longhine M: Effect of an interleukin-4 variant on late phase asthmatic response to allergen challenge in asthmatic patients: results of two phase 2a studies. Lancet 2007, 370:1422–1431.
23. Ghoreschi K, Rocken M: Immunopathogenesis of psoriasis. J Dtsch Dermatol Ges 2003, 1:534–532.
24. Romer PS, Berr S, Avota E, Na SY, Battaglia M, ten Berge I, et al: The role of PBMC at high cell density increases sensitivity of T-cell responses, revealing cytokine release by CD28 superagonist TGN1412. Blood 2011, 118:6772–6782.
25. LaPorte SL, Juo ZS, Vaclavikova J, Colf LA, Qi X, Heller NM, et al: Inhibition of CD23 processing correlates with inhibition of IL-4-stimulated IgE production in human PBL and hu-PBL-reconstituted SCID mice. Clin Exp Allergy 2009, 39:250–259.
26. Burmeister Getz E, Fisher DM, Fuller R: Human pharmacokinetics/pharmacodynamics of an interleukin-4 and interleukin-13 dual antagonist in asthmatic mice. J Clin Pharmacol 2004, 44:245–261.
27. Kobayashi S, Haruo N, Sugane K, Ochs HD, Agematsu K: Interleukin-21 stimulates B-cell immunoglobulin E synthesis in human beings concomitantly with activation-induced cytokine deaminase expression and differentiation into plasma cells. Hum Immunol 2009, 70:35–40.
28. Mayer RJ, Bolognese BJ, Al-Mahdi N, Cook RM, Flammberg PL, Hansbury MJ, et al: Site-directed mutagenesis reveals the importance of disulfide bridges and aromatic residues for structure and proliferative activity of human interleukin-4. J Exp Med 1994, 205:719–727.
29. Kaminishi K, Soma Y, Kaya W, Mizooguchi M: Flow cytometric analysis of IL-4, IL-13 and IFN-gamma expression in peripheral blood mononuclear cells and detection of circulating IL-13 in patients with atopic dermatitis provide evidence for the involvement of type 2 cytokines in the disease. J Dermatol Sci 2002, 29:19–25.
30. Obara W, Kaya Y, Ra C, Nishioka K, Soma Y, Mizooguchi M: T cells and mast cells as a major source of interleukin-13 in atopic dermatitis. Dermatology 2002, 205:11–17.
31. Kaminishi K, Soma Y, Kaya W, Mizooguchi M: Flow cytometric analysis of IL-4, IL-13 and IFN-gamma expression in peripheral blood mononuclear cells and detection of circulating IL-13 in patients with atopic dermatitis provide evidence for the involvement of type 2 cytokines in the disease. J Dermatol Sci 2002, 29:19–25.
32. Spiegelberg HL, Beck L, Kocher HP, Fanslow WC, Lucas AH: Interleukin 4 or cytokine antagonists: time to change the search for novel psoriasis therapies. Dermatology 2010, 221:27–29.
33. Biedermann T, Zimmermann S, Himmelrich H, Gumy A, Egeter O, Sakrauski et al: Cite this article as: Zadeh-Khorasani et al: NOD-scid IL2Rnull mice engrafed with human peripheral blood mononuclear cells as a model to test therapeutics targeting human signaling pathways. Journal of Translational Medicine 2013 11:4. doi:10.1186/1479-5876-11-4.