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Modulation of the allergen-induced human IgE response in Hu-SCID mice: inhibitory effect of human recombinant IFN-gamma and allergen-derived lipopeptide

Abstract

We have previously established a model to study the in vivo human IgE response using humanized SCID mice. Allergic SCID mice were obtained following intraperitonal injection with mononuclear cells from Dermatophagoides pteronyssinus (Dpt)-sensitive patients, and sensitization by Dpt allergen

intraperitonal injection (immunization) or Dpt aerosol (inhalation). Human serum IgE was measured in allergic SCID mice after administration of human recombinant IFN-gamma or the lipopeptide LP 52-71 (derived from peptide p52-71 from Der p 1, Dpt major allergen, coupled to a lipophilic moiety), during the immunization or the inhalation phase. IFN-gamma inhibited human IgE production when given at the time of immunization, but not during inhalation. This effect was long-lasting as Dpt aerosol, given one month after immunization and IFN-gamma administration, failed to increase IgE levels. Unlike Dpt or p52-71, LP 52-71 failed to induce human IgE production at day 14 and 21 after its injection, but did inhibit the development of the IgE response after a secondary Dpt-challenge. Moreover, LP 52-71 administration 14 days after Dpt inhalation decreased IgE levels, in contrast to peptide 52-71, which increased IgE levels. Thus, taken together these results indicate that the development of the human IgE response in allergic SCID mice can be modulated by modified allergen and a Th1 cytokine.

Full text

INTRODUCTION

Many studies have been devoted to understanding the mechanisms of the human allergic reaction, which is mainly characterized by an enhanced IgE production and a local inflammatory reaction. The IgE response is dependent on several factors including genetic background and conditions of exposure (dose of allergens, timing and route of allergen introduction) [1, 2]. The IgE production is also directly dependent on the nature of the antigens and on the level of Th2/Th1 cytokine production. Indeed, although a variety of cytokines may be implicated in the T cell-dependent regulatory process, the balance between main antagonistic cytokines IL-4 (Th2) and IFN-gamma (Th1) appears to be one of the key determinants in the *in vivo* expression of the IgE-responder phenotype.

Several reports indicate that IFN-gamma can modulate human IgE production. IFN-gamma has been demonstrated to inhibit IL-4-dependent IgE synthesis by reducing the expression of mRNA encoding for the constant region of IgE [3]. The role of IFN-gamma-producing-$CD4^+$ T cells in the induction of the Th0 or Th1 type response is well documented [4]. After *in vitro* restimulation, antigen-specific T cell clones, producing IFN-gamma, fail to induce an IgE response, but

increase the IgG response [5, 6]. In various animal models, data emphasize the pivotal role of this cytokine [7, 8]. For example, after repeated exposure to an aerosol of ovalbumin (OVA), the high-IgE responder Brown-Norway rats developed a transient, OVA-specific IgE response; the subsequent decrease in IgE production was associated with an increase in IFN-gamma production [9]. Similar results were reported in mice repeatedly exposed to allergen aerosol [10, 11]. This decrease in IgE secretion was due to the negative regulatory effect of IFN-gamma produced mainly by $CD8^+$ T cells.

Other attempts to inhibit IgE production have been made using modified allergen epitope in order to skew the immune response towards the IFN-gamma-associated Th1 phenotype. Different methods of chemical modification such as polymerization, denaturation and conjugation of allergens with a variety of carriers have been tested [12-14]. Such chemical modifications may allow reduction in either the frequency of allergen injections during immunotherapy or the allergenicity, and may increase the ability to induce tolerance to the allergen. Moreover, the fact that liposome-associated allergen might reduce the initiation as well the ongoing IgE response has opened another possibility of using major T or B cell epitopes coupled to a lipophilic moiety in order to downregulate the IgE response. Indeed, lipopeptides derived from influenza virus were shown to prime influenza virus-specific cytotoxic T lymphocytes *in vivo* as efficiently as the infectious viruses did. More recent experiments with peptides derived from human immunodeficiency virus envelop glycoprotein, from the foot and mouth virus or from the malaria parasite indicated that lipopeptides were also efficient at stimulating a $CD4^+$ T cell-dependent antibody response without requiring adjuvant [15-17]. Moreover, liposome formulation of a peptide of the hepatitis B core antigen induced a Th1 response, in contrast to the antigen alone, which leads to a Th2 response in mice [18].

Among the aeroallergens, the house dust mite *Dermatophagoïdes pteronyssinus* (Dpt) is an important cause of allergic disorders. Der p 1, a 222-amino acid glycoprotein, is one of the major allergenic molecules of Dpt. Different approaches have been used to map B and T cell epitopes of Der p 1. Using conventional predictive methods and a tridimensional model of Der p 1, four peptides were selected as potential B cell epitopes and were shown to exhibit IgE-dependent biological activities [19, 20]. In this study, one of them, peptide p52-71, was modified by adding a lipid moiety in order to avoid its

tendency to favor an IgE response, and its efficacy was analyzed in the model of the humanized SCID (severe combined immunodeficient) mouse.

Due to a mutation affecting their recombinase system leading to a deficiency in mature T and B cells, SCID mice tolerate normal human lymphoid tissues (including fetal liver, bone marrow, and thymus), and allow to some extent the differentiation of human lymphoid cells. Moreover, mature human lymphocytes isolated from peripheral blood survive for extended periods of time in SCID mice and develop a spontaneous production of human immunoglobulin. When human peripheral blood cells from donors vaccinated against tetanus toxoid are used for mice reconstitution, the immunized Hu-SCID mice develop an antigen-dependent antibody response against the related antigen [21]. In our laboratory, SCID mice, reconstituted with purified peripheral mononuclear cells from donors sensitive to house dust mite allergens (*Dermatophagoides pteronyssinus* or Dpt), were shown to develop a specific human IgE response after either intraperitoneal injection of allergen in the presence of adjuvant, or exposure to allergen aerosol [22, 23]. Thus, this SCID model appears appropriate to further analyze the potential regulatory factors involved in the control of the human IgE response. Indeed, this report provides evidence that recombinant IFN-gamma and Der p 1-derived lipopeptides can reduce *in vivo* human IgE production.

MATERIALS AND METHODS

Mice

C.B.-17 SCID mice were maintained in sterilized isolators (La Calhène, France) with sterilized bedding, at the Pasteur Institute of Lille.

Human peripheral blood mononuclear cells (PBMC)

Blood was collected from donors sensitive or non-sensitive to house dust mite. Allergic patients presented usual features of house dust mite sensitization: positive skin prick-tests for *Dermatophagoides pteronyssinus (Dpt)* allergen (Stallergènes, France), positive RAST (Pharmacia, Sweden) (class 2 to 4). Serum IgE levels of the allergic patients ranged from 400 to 1,814 IU/ml. Healthy, non-allergic donors (*Dpt* RAST < 0), (without any allergic disorder or atopy), were tested as negative controls. PBMC from blood from donors sensitive (n = 12) (four positive donors were twice involved in different experiments) or non-

sensitive (n = 5) to *Dpt* were prepared by density centrifugation on Ficoll-Paque (Pharmacia, France). Mononuclear cells harvested at the interface were washed three times in sterile RPMI medium before transfer.

Cell transfer and immunization

Six to 8 week-old SCID mice were reconstituted by intraperitoneal (ip) injection of 10 to 15 x 10^6 cells in 200 mul of RPMI using a 23-gauge needle. The number of PBMC from each donor was variable and allowed reconstitution of 6 to 12 mice. For some experiments, one day prior to reconstitution, SCID mice were treated with 50 mul ASGM1 antibody (Wako Chemicals, Neuss, Germany) to enhance cell engraftment [24]. Two different sensitization protocols were used (Figure 1). For protocol 1, mice were immunized by ip injection with a low dose of Dpt allergen (Stallergènes, France) (5 IR/ml) in aluminum hydroxide-Alu-Gel-S (Serva, Heidelberg, Germany) at D0, two weeks after cell transfer. For protocol 2, SCID mice were ip injected with 2IR Dpt allergen on the day of reconstitution, and four days later (D0) were exposed to Dpt aerosols for 4 consecutive days (D0 to D3). Reconstituted hu-SCID mice were placed in a sterilized air flow-controlled chamber connected to a nebulizer (VarioSonic Peters, Aubervilliers, France) generating 4-6 mum size droplets, and were exposed to Dpt diluted in saline (10 IR/ml) for 30 min. The inhalation step, which took place outside of the isolators where mice were housed, occurred in a sterilized plexiglass chamber in which the airflow was controlled.

IFN-gamma *administration*

Recombinant human IFN-gamma (Tebu, France) was either injected ip at day (D) 0, D2, D7 (100 ng/mouse) or administered using osmotic pumps (Alzet) placed under mouse skin for seven days, which delivered a total of 200 ng/ml (from D1 to D7). All animals received an additional ip injection of IFN-gamma at D10.

Preparation of Der p1 and Der p 1-derived lipopeptide

Immunoaffinity purified Der p 1 was prepared as previously reported [25]. Endotoxin contamination was always below 12.5 pg/ml. One peptide derived from Der p 1 allergen, peptide p52-71 NQSLDLAEQELVDCASQHGC was synthesised using a solid phase method and coupled to a palmitoyl moiety as previously described for other peptides [26]. The homogeneity of the peptide was checked

by analytical reversed phase-HPLC, and the amino-acid composition verified by total acid hydrolysis.

Synthetic lipopeptide administration

The effect of the lipopeptide was evaluated in 2 different systems. Firstly, using protocol 1, SCID mice were injected with one dose (25 mug/mouse) of lipopeptide instead of Dpt allergen or Der p 1 peptide, at D0. Alternatively, in accordance with protocol 2, humanized SCID mice were exposed to Dpt aerosol, and at D14, after allergen inhalation, were injected ip with the lipopeptide LP 52-71.

Human IgE measurement

After reconstitution, mice were periodically bled from the retro-orbital sinus under ether anesthesia. Total human serum IgE was detected by a fluorometric method (CAP system, Pharmacia, Sweden), using two different mouse monoclonal antibodies specific for the epsilon-chain. The sensitivity of the method was 0.1 IU/ml (0.24 ng/ml).

Statistical analysis

Statistical analysis was performed using the Statview F4.11 Software (Apple Company, Copertino, Calif., USA). Comparisons were performed using the Mann-Whitney test. p values for significance were set at 0.05. Values for all measurements are expressed as the mean ± standard error of the mean (SEM).

RESULTS

Effect of human IFN-gamma *on human IgE production in SCID-mice reconstituted with PBL from Dpt-sensitive patients*

SCID mice were reconstituted with 10-15 x 10^6 peripheral blood mononuclear cells from four different donors sensitive to Dpt. Two weeks after cell reconstitution, all the humanized SCID mice were immunized ip with allergen, in the presence of alum as adjuvant (Protocol 1). These mice produced human IgE reaching a maximum at D21 and D28 (Figure 2). At the time of immunization (D0) and on D4, D7 and D10, some humanized SCID mice were injected ip with

recombinant human IFN-gamma. Such treatment significantly reduced human IgE production, mainly at Day 21 (Figure 2A). Similar results (with PBMC from two other donors) were obtained using osmotic pumps delivering human IFN-gamma from D0 to D7, followed by an additional ip injection of IFN-gamma, at D10 (Figure 2B). In contrast, when SCID mice were reconstituted and then exposed to Dpt by the inhalation route (Protocol 2), ip injection of recombinant human IFN-gamma at the time of inhalation, failed to significantly decrease IgE production (Figure 3). When SCID mice were reconstituted with PBMC from donors not sensitive to Dpt, no siginificant IgE production was demonstrated using protocol 1 as previously described [22, 23] or protocol 2 and in neither case did IFN-gamma modify this effect (data not shown).

The immunomodulatory effect of human IFN-gamma was more evident when humanized SCID mice, previously treated or not with IFN-gamma at the time of immunization, were subsequently challenged with Dpt aerosol one month later (Figure 4). Indeed, one month after ip immunization with Dpt, when the group of humanized SCID mice was challenged with two Dpt aerosols, the level of IgE production significantly increased for each mouse. In contrast, humanized SCID mice pretreated with IFN-gamma at the time of immunization (D0), did not increase their IgE production when secondarily challenged with Dpt aerosol (Week 0). These results indicate that human IFN-gamma can modulate the human IgE response in humanized SCID mice.

Effect of the Der p 1-derived lipopeptide on human IgE production in Hu-SCID-mice

As lipopeptides have been recently described as potential inducers of the protective immune response, the efficacy of the Der p 1 lipopeptide (LP 52-71) in humanized SCID mice was tested. When SCID mice were reconstituted with peripheral mononuclear cells from donors allergic to Dpt, and two weeks later (D0) received the related allergen in the presence of alum adjuvant intraperitoneally (protocol 1), a significant human IgE production was evident within the next three weeks. The injection at D0, of the synthetic peptide p52-71, known as a potential B cell epitope in the presence of alum adjuvant, also induced an intense IgE response. However, when this synthetic peptide was coupled to a lipid moiety (lipopeptide LP52-71) and injected ip in the presence of alum adjuvant at D0, the IgE production was significantly reduced compared to Dpt or ip injection of p52-71 (Figure 5). The effect of lipopeptide was not tested

in control SCID mice (reconstituted with PBMC from healthy donors), because no IgE was demonstrated.

SCID mice, humanized with PBMC from Dpt sensitive donors, and previously injected with Dpt or Der p 1 peptide p52-71 (D0), developed a further human IgE production after a secondary challenge by ip Dpt injection one month later. In contrast, treatment with lipopeptide LP 52-71 (D0), followed by the secondary ip Dpt injection (one month later) failed to increase the human IgE production two weeks later (Figure 6).

Moreover, the lipopeptide LP 52-71 was demonstrated to reduce the ongoing response. Humanized SCID mice exposed to Dpt aerosol (D0) (protocol 2) and subsequently injected ip at D14 with the lipopeptide LP 52-71, failed to increase their IgE production, in contrast to the group of SCID mice injected with the related synthetic peptide p52-71 on D28 (Figure 7). Alternately, injection of LP 52-71, at D14, decreased the IgE response at D28 compared to non-treated animals. Taken together, these results emphasize the negative regulatory effect of Der p 1 lipopeptide LP52-71 on the human IgE induced in humanized SCID mice.

DISCUSSION

In this study, we have demonstrated for the first time that human IgE production induced in SCID mice, reconstituted with peripheral blood cells from patients sensitive to *Dermatophagoides pteronyssinus* (Dpt), is significantly reduced following two different procedures: administration of recombinant IFN-gamma or of a lipopeptide derived from Dpt major allergen, Der p 1.

The negative regulatory effect of IFN-gamma was observed when administered to Hu-SCID mice at the time of allergen immunization (D0), by ip injection of Dpt in the presence of alum adjuvant (Protocol 1). It may be postulated that, in the presence of adjuvant, the allergen present in the peritoneal cavity stimulates allergen specific T cells to produce cytokines, and in particular Th2 cytokines favored by the presence of alum adjuvant. Thus, the injection of recombinant human IFN-gamma, starting at D0, is able to interfere and compete with the IL-4-dependent mechanisms and decreases the possibility of allergen-specific B cells efficiently switching to the IgE isotype. Subsequent local injections of rIFN-gamma on the following days might amplify the negative regulatory effect on IgE production. As IFN-gamma has been reported to have no direct effect on human

purified B cell populations [4], the potential target of rIFN-gamma could be $CD4^+$ T cell subsets. IFN-gamma inhibits the proliferative response of Th2 cells and the IL-4-dependent function of Th2 cells [27], but upregulates its own expression and enhances the pro-Th1 IL-12 cytokine effect. The importance of cytokines in $CD4^+$ T cell differentiation has been illustrated by a number of experiments in which the presence of a cytokine at the time of initial antigen exposure may modify the subsequent functional Th subset induced [28, 29]. The effect of human rIFN-gamma appeared to be long lasting. Indeed, when Hu-SCID mice have been previously treated with recombinant human IFN-gamma, they failed to produce an IgE response after allergen challenge given either intraperitoneally or as an aerosol, suggesting that the Th1 cytokine environment prevented allergen-induced Th2 responses.

In contrast, when administered ip at the time of inhalation (protocol 2), rIFN-gamma did not inhibit the development of the IgE response. Indeed, in classical animal models, IFN-gamma has to be given before the first allergen administration to inhibit the IgE response [30], but does not affect total IgE level in serum, if given after immunization and at the time of inhalation [31]. In protocol 2 of our study, mice were injected ip with Dpt at the time of reconstitution, and were ip injected with IFN-gamma 4 days later, at the time of the Dpt aerosol. IFN-gamma is therefore given after B cells start switching to produce IgE, and could have been introduced too late to inhibit this Th2-dependent mechanism. Moreover, as the administration of Dpt aerosol was undertaken for four days, the specific-antigen T and B cells migrating towards antigen could escape the effect of IFN-gamma given intraperitoneally.

However, administration of human recombinant IFN-gamma was performed in each case (protocol 1 or 2) after the initial allergen challenge that occurs *in vivo* in the patient. In protocol 1, Dpt was injected in the presence of alum as adjuvant, which could be strong enough to drive new switches of B cells towards IgE production. IFN-gamma given at that time, could therefore block these new switches, and effectively decrease IgE production. In contrast, in protocol 2, Dpt injection is given without alum at the time of inhalation and could specifically activate patients' Dpt-specific T cells without being able to drive other B cells' switch. Attempts to modulate the IgE response by different cytokines have been already reported in SCID mouse reconstituted with PBMC from allergic patients. However, the difference in our protocol, in which moderately allergic patients were used, showed that when mononuclear cells from atopic donors producing

high IgE level were used to reconstitute SCID mice, even large amounts of IFN-gamma failed to reduce IgE production [32]. In contrast to IL-4, the effect of IFN-gamma on the B cell population is not direct and requires the involvement of T cells. In this case, the B cells from atopic patients are already committed, and some B cells escaped the effect of regulatory cytokines. In our model, the B cells came from moderately allergic patients secreting moderate levels of IgE and some of them had not already switched to produce IgE; this induction of IgE production requires the participation of Th2 cells and of Dpt allergen. Nonetheless, in humans suffering from atopic dermatitis, where the allergen responsible might yet be present, the efficacy of the *in vivo* administration of IFN-gamma has been discussed [33]. Thus, recombinant IFN-gamma can interfere with the IL-4-dependent mechanism of the IgE production sequence induced by allergen in humanized SCID mice.

In another attempt to modulate the human IgE response in SCID mice, we evaluated the effect of the injection of p52-71, peptide derived from the Dpt major allergen, Der p 1, and of LP52-71, lipopeptide constructed with p52-71 coupled to a palmitoyl moiety. Injection of p52-71 in the presence of alum as adjuvant, induced IgE production at the same level as Dpt injection. Der p 1 synthetic peptide, p52-71, has been previously identified as a potential B cell epitope on the basis of its capacity to activate basophils ands platelets from Dpt-sensitive patients. Moreover, p52-71 has been found to be immunogenic in rat and rabbit, *i.e.* able to induce T cell-dependent B cell activation [34]. However, intranasal administration of p111-139, a major T cell epitope of Der p 1, followed by Der p 1 subcutaneous injection, induced tolerance in mice [35]. Indeed, *ex vivo* proliferation of lymph node-derived-T cells and specific IgE production were inhibited by *in vivo* p111-139 treatment. Differences in peptide administration (intranasally without adjuvant *versus* intraperitonally with adjuvant), as well as peptide size, could explain the differences in the modulation of IgE production (inhibition *versus* induction). Oral or subcutaneous administration of peptides was also shown to inhibit the activation of allergen-specific T cells. Indeed, naive mice pretreated with peptides of Der p 1 or Fel d 1 did not develop a cellular immune response when challenged with native allergen. In addition, ongoing immune response to native Der p 1 or Fel d 1 allergen could be downregulated by peptide treatment (reviewed in [36]). However, clinical trials with Fel d 1-derived peptides did not lead to significant reduction of the allergic parameters or to a definitive shift towards the Th1 phenotype [37].

As regards the role of lipopeptides, this is, to our knowledge, the first study indicating that lipopeptides can modulate the IgE response. Mechanisms by which lipopeptides can modulate the immune response are still debated. Several studies have shown enhanced major histocompatibility complex (MHC) class I restricted presentation to cytotoxic T lymphocytes (CTL) [16, 38]. The lipid moiety is thought to either increase the time of peptide availability *in vivo*, or induce a selective entry of the lipopeptide into the MHC class I processing pathway through membrane attachment, or to activate macrophages, or increase MHC class II restricted presentation to T helper cells, required for the generation of CTL. Other studies have shown that lipopeptides could induce a T helper cell response [39, 40]. In this study, we have shown that ip LP52-71 injection, in the presence of adjuvant, does not induce IgE production, in contrast to p52-71 injection. Several hypotheses could explain this inhibition. First, palmitoyl-coupled peptide LP52-71 could induce a CTL response, therefore inhibiting the T helper response. The palmitoyl group has been specifically shown to allow a rapid and passive membrane translocation of the associated peptide, inducing peptide presentation to CTL [38]. Second, LP52-71 could elicit a Th1 response instead of a Th2 response involved in IgE production. Indeed, immunization of mice with a lipopeptide derived from a major surface glycoprotein of *Leishmania major*, induced a Th1 response associated with cell-mediated immunity [40]. In our study, the modifications of the IgE response obtained after treatment with lipopeptide LP 52-71, were similar to some extent, to those observed with IFN-gamma, supporting this hypothesis, and suggesting the importance of antigen-specific T cells in this SCID model of allergic reaction. Mechanisms involved in the reduction of human IgE production followed by LP52-71 administration in allergic SCID mice, have yet to be explored.

Thus, by using allergic reconstituted SCID mice, the human IgE response could be modulated either by the Th1 cytokine IFN-gamma or by a lipopeptide derived from a peptide coupled to a lipid moiety, supporting the potential of SCID mice as a model for better understanding of the mechanisms controlling human allergic reactions. In addition, this study shows the efficacy of a lipopeptide derived from house dust mite to reduce *in vivo* human IgE production, suggesting the importance of using such molecules in the treatment of IgE-mediated allergic reactions.

CONCLUSION

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Figures

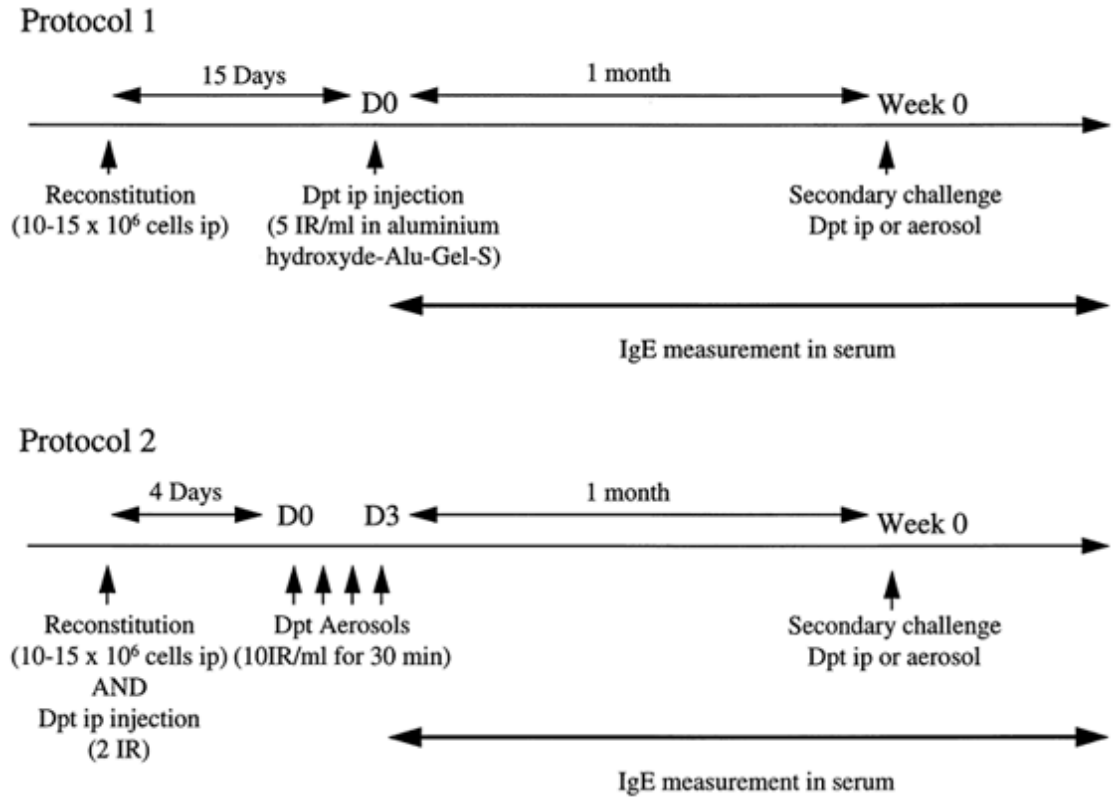
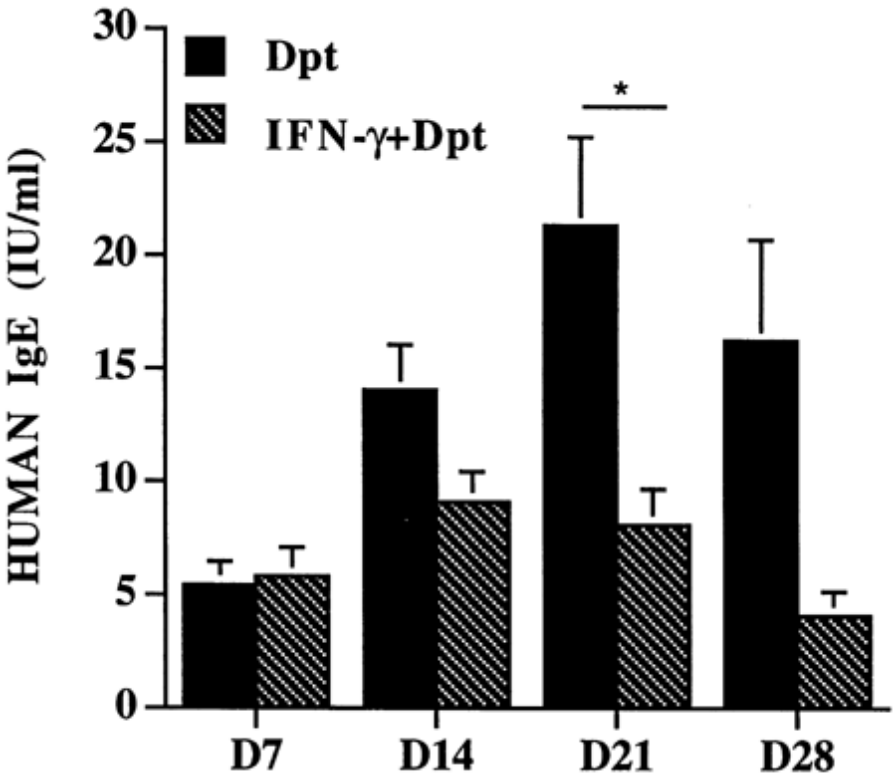


Figure 1
Immunization (protocol 1) or inhalation (protocol 2) protocols.



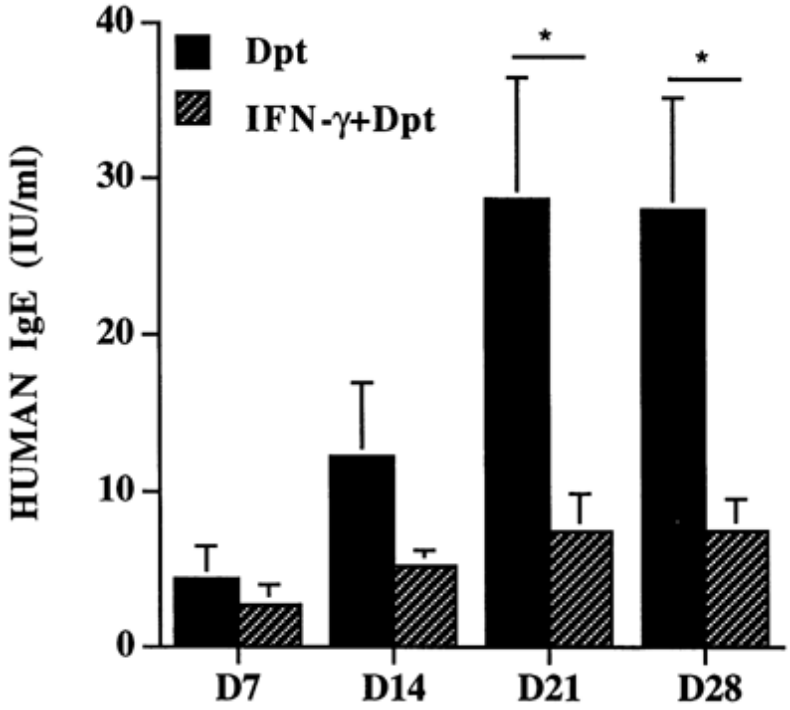

HUMAN IgE (IU/ml)
40
30
20
10
0
Dpt
IFN-γ+Dpt
*
*
D7
D14
D21
D28

Figure 2
Effect of recombinant human IFN-gamma on the human IgE production induced after Dpt injection (protocol 1).

SCID mice were reconstituted with 10-15 x 10^6 PBMC from six patients sensitive to *Dermatophagoides pteronyssinus* (Dpt), and two weeks later (at D0) were injected intraperitoneally with Dpt allergen (SA Stallergenes) (5 IR/ml) in the presence of alum as adjuvant. Recombinant human IFN-gamma (100 ng/mouse) was injected ip at D0, D2, D7 and D10 to reconstituted SCID mice (n = 16) **(A)** or administered with micro-osmotic pumps (200 ng/mouse) for 7 days (D1 to D7) followed by a single ip injection (100 ng/mouse) at D10 (n = 7) **(B)**. Levels of human IgE were evaluated within the next 30 days after allergen injection and compared to control mice (2A: n = 12, 2B: n = 5) not treated with IFN-gamma. Results are expressed as mean ± SEM. *: $p < 0.05$ according to the Mann-Whitney test.

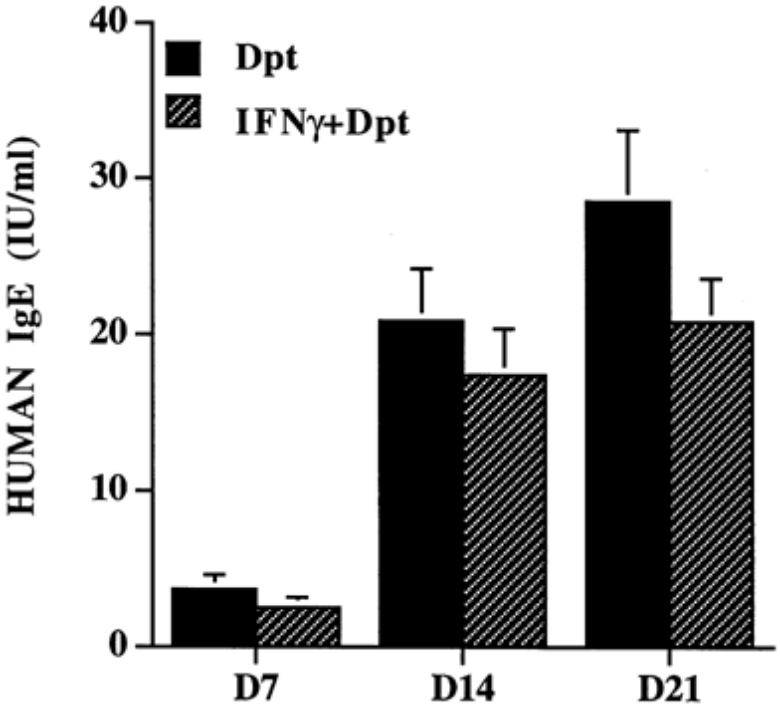


Figure 3
Effect of recombinant human IFN-gamma on the IgE response induced by allergen inhalation (protocol 2).

SCID mice were reconstituted with PBMC from three patients sensitive to Dpt and received 2 IR of Dpt by ip injection on the day of cell reconstitution. Four days later (D0) SCID mice were exposed to four Dpt aerosols (from D0 to D3). Reconstituted SCID mice were intraperitoneally injected at D0, D2, D7 and D10 with recombinant human IFN-gamma (n = 10) or saline (n = 8). Human IgE produced by reconstituted SCID mice was evaluated after Dpt allergen inhalation. Results are expressed as mean ± SEM.

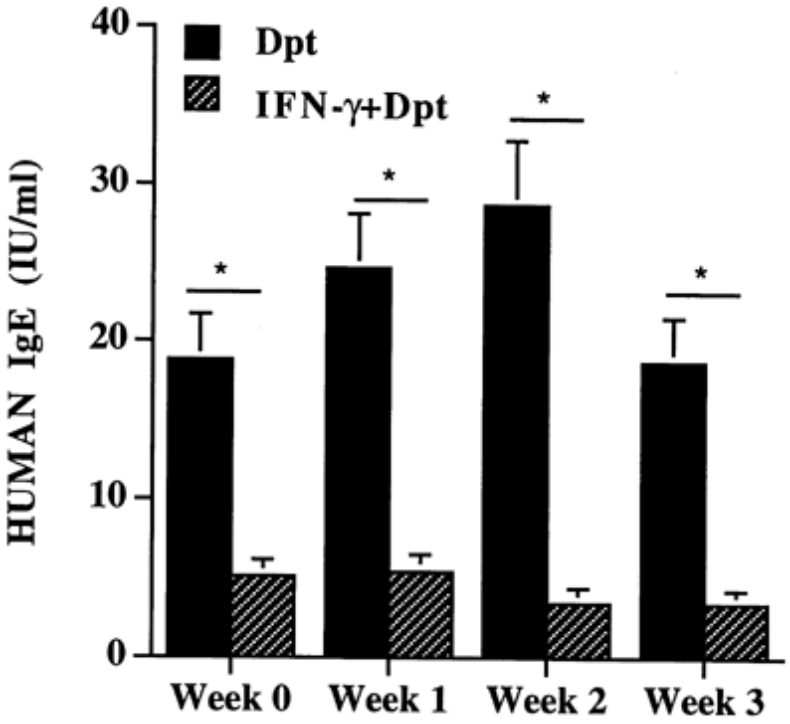


Figure 4
Effect of recombinant human IFN-gamma on secondary IgE production.

SCID mice were reconstituted and received intraperitoneal injection of Dpt alone (n = 8) or Dpt and IFN-gamma (n = 8) (protocol 1). One month later, reconstituted SCID mice were exposed to Dpt aerosol as a secondary challenge and IgE production was followed over a period of three weeks after the secondary allergen challenge. Results are expressed as mean ± SEM. *: $p < 0.05$ according to the Mann-Whitney test.

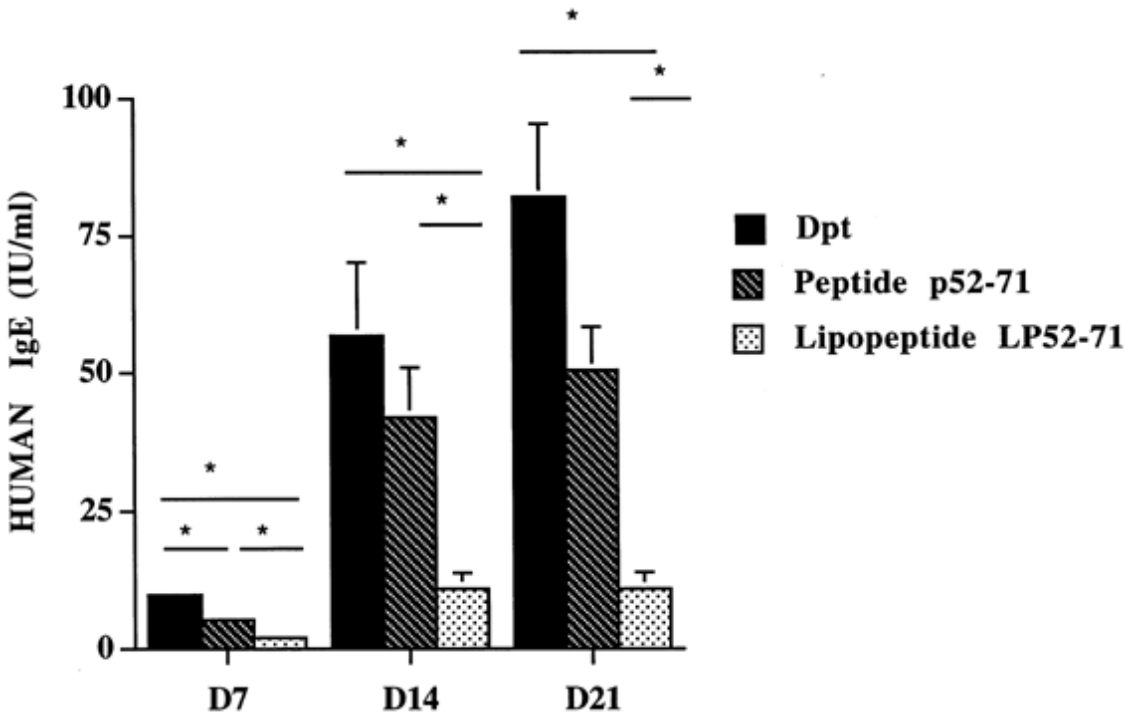


Figure 5
Effect of Der p 1-derived peptide p52-71 or lipopeptide LP 52-71 on IgE production in reconstituted SCID mice.

After a pretreatment with the anti-asialoGM1 antibody (D-1) in order to promote cell reconstitution, SCID mice were reconstituted (with PBMC from 2 patients allergic to Dpt) and immunized by ip injection with either Dpt (n = 5), Der p 1 peptide 52-71 (n = 4), or lipopeptide LP 52-71 (n = 6) in the presence of alum (protocol 1). Results are expressed

as mean ± SEM. *: p < 0.05 according to the Mann-Whitney test.

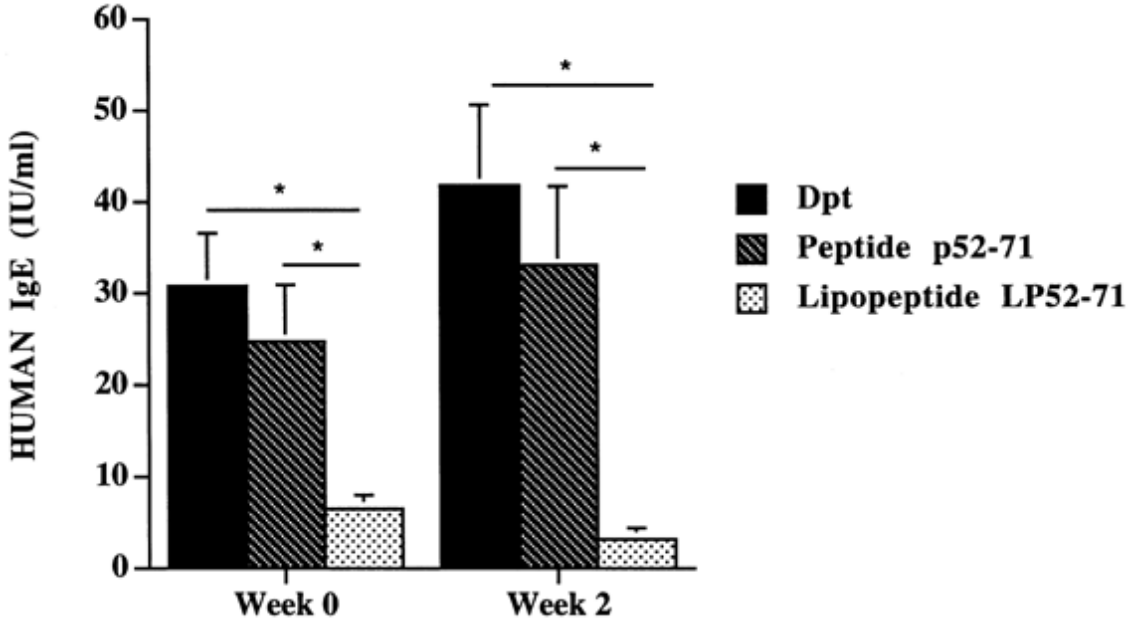


Figure 6
Pretreatment with the lipopeptide LP52-71 prevents IgE production induced after a secondary Dpt intraperitoneal allergen challenge.

Two weeks after reconstitution (with PBMC from 3 patients allergic to Dpt) (protocol 1), SCID mice received ip injection of either Dpt (n = 9), peptide p52-71 (n = 4) or lipopeptide LP 52-71 (n = 10). One month after sensitization (D0), both groups of SCID mice were challenged by an intraperitoneal injection of Dpt. Results are expressed as mean ± SEM. *: p < 0.05 according to the Mann-Whitney test.

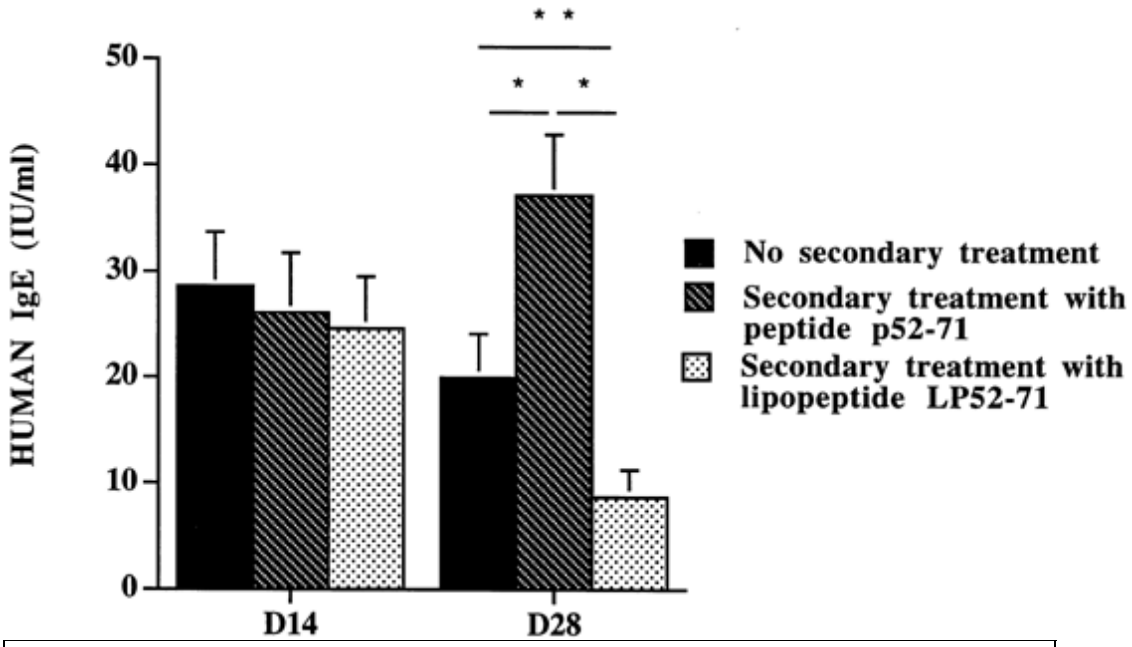


Figure 7
Effects of the lipopeptide LP52-71 on the ongoing human IgE response induced in reconstituted SCID mice.

Humanized SCID mice (with PBMC from 2 patients allergic to Dpt) were injected one day before cell reconstitution with ASGM1 antibody to improve the reconstitution. On the day of cell reconstitution, SCID mice were injected ip with Dpt allergen (2 IR/mouse) and four days later were exposed to four Dpt aerosols from D0 to D3. At day 14, SCID mice were either non-treated (n = 6), or injected intraperitoneally either with peptide 52-71 (n = 7) or lipopeptide LP 52-71 (n = 7). Human IgE was evaluated at D14, D28 after the exposure to Dpt aerosols. Results are expressed as mean ± SEM. *: $p < 0.05$ according to the Mann-Whitney test.

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In this issue

Virus-cell interactions: impact on cytokine production, immune evasion and tumor growth

T. H. Mogensen, S. R. Paludan
virus, cytokines, signal transduction, immune evasion, tumor growth.

Safety, pharmacokinetics and pharmacodynamics of recombinant human tumour necrosis factor-binding protein-1 (Onercept) injected by intravenous, intramuscular and subcutaneous routes into healthy volunteers

I. Trinchard-Lugan, Q. Ho-Nguyen, W.M. Bilham, M. Buraglio, A. Ythier, A. Munafo
recombinant human tumour necrosis factor-binding protein-1 (r-hTBP-1), safety, pharmacokinetics, pharmacodynamics.

Direct effects of tumor necrosis factor alpha (TNF-alpha) on murine skeletal muscle cell lines. Bimodal effects on protein metabolism

B. Alvarez, L. S. Quinn, S. Busquets, F. J. López-Soriano, J. M. Argilés
cytokines, myotubes, cachexia, protein turnover.

Receptor-like properties of the 26 kDa transmembrane form of TNF

A. Domonkos, A. Udvardy, L. László, T. Nagy, E. Duda
cytokine, TNF family, receptor, signaling, reverse-signaling.

Local administration of interleukin-1beta for the treatment of lung abscesses induces neutrophil activation and changes in proinflammation cytokine production

A. Simbirtsev, E. Variouchina, V. Konusova, A. Kotov, S. Ketlinsky, A. Salamatov, L. Bisenkov

recombinant human interleukin-1beta, lung inflammation, abscess, neutrophils, cytokines.

Effects of interleukin-2 therapy on the proliferation and differentiation of CD4/CD25 positive and CD4/CD25 negative cells in HIV+ patients

L. Caggiari, S. Zanussi, M. D'Andrea, M. T. Bortolin, C. Crepaldi, C. Caffau, P. de Paoli
cytokines, Ki67, intracellular IFN-gamma.

Tumor necrosis factor-alpha and nitric oxide in vertically HIV-1-infected children: implications for pathogenesis

J. González-Nicolás, S. Resino, J. L. Jiménez, S. Alvarez, M. Fresno, M. A. Muñoz-Fernández
tumor necrosis factor, nitric oxide, HIV-infection, immunological and virological markers.

Serum levels of angiogenic cytokines in systemic lupus erythematosus and their correlation with disease activity

E. Robak, A. Wozniacka, A. Sysa-Jedrzejowska, H. Stepien, T. Robak
systemic lupus erythematosus, disease activity, angiogenesis, platelets, hepatocyte growth factor, vascular endothelial growth factor, basic fibroblast growth factor, transforming growth factor beta.

Modulation of the allergen-induced human IgE response in Hu-SCID mice: inhibitory effect of human recombinant IFN-gamma and allergen-derived lipopeptide

C. Duez, H. Gras-Masse, H. Hammad, H. Akoum, A. Didierlaurent, C. André, A.-B. Tonnel, J. Pestel
SCID mouse, IgE, IFN-gamma, lipopeptide, house dust mite.

Cytokine-inducing activity of a proline-rich polypeptide complex (PRP) from ovine colostrum and its active nonapeptide fragment analogs

A. Zablocka, M. Janusz, K. Rybka, I. Wirkus-Romanowska, G. Kupryszewski, J. Lisowski
ovine colostrum, proline-rich polypeptide complex (PRP), nonapeptide (NP) and hexapeptide (HP) from PRP, oligomers of NP and HP, cytokines.

Quantitation of chemokines (MDC, TARC) expression in mucosa from Crohn's disease and ulcerative colitis

F. Jugde, M. Alizadeh, C. Boissier, D. Chantry, L. Siproudhis, S. Corbinais, E. Quelvennec, F. Dyard, J.-P. Campion, M. Gosselin, J.-F. Bretagne, G. Sémana, D. Heresbach
real-time TR-PCR, chemokines, Crohn's disease, ulcerative colitis.

Activation of caspase-3 by interferon alpha causes interleukin-16 secretion but fails to modulate activation induced cell death

O. Ludwiczek, A. Kaser, R. O. Koch, W. Vogel, W. W. Cruikshank, H. Tilg
interleukin-16, interferon-alpha, caspase-3.

Cytokine deviation induced by intrathymic injection of staphylococcal enterotoxin B (SEB)

P. Goettelfinger, F. Lecerf, S. Berrih-Aknin, M. German-Fattal
SEB, intrathymic injection, anergy, cytokine deviation, quantitative RT-PCR.

Strain-dependent difference in inducible nitric oxide synthesis (iNOS) expression in rat pancreatic islets correlates with interferon regulating factor 1 (IRF-1) and heat shock protein 70 (HSP70) expression

J. Johannesen, F. Pociot, A. E. Karlsen, T. Mandrup-Poulsen, J. Nerup
type 1 diabetes, islets of Langerhans, beta-cells, cytokines, interleukin-1beta, interleukin-1beta receptor type I, nitric oxide, inducible nitric oxide synthase, heat shock protein 70, interferon regulating factor 1.

Neutrophil apoptosis in autoimmune Fas-defective MRL lpr/lpr mice

E. Del Giudice, A. Ciaramella, N. Balestro, D. Neumann, P. G. Romano, M. P. Cesaroni, G. Maurizi, P. Ruggiero, D. Boraschi, P. Bossù
neutrophils, apoptosis, autoimmunity.

A role for interferon-gamma in the hypermetabolic response to murine toxoplasmosis

D. Arsenijevic, F. de Bilbao, P. Giannakopoulos, L. Girardier, S. Samec, D. Richard
toxoplasma, hypermetabolism, reactive oxygen species, lipid peroxidation, UCP1, interferon-gamma.

Protection of mice from malaria after co-administration of recombinant mouse granulocyte-macrophage colony-stimulating factor and methionine-enkephalin

P. Pal Singh, S. Singh
Plasmodium berghei, protection, rmGM-CSF, met-enkephalin.

Semiautomated and simultaneous analysis of the interleukin-10 gene microsatellites IL-10G and IL-10R by fluorescence-based polymerase chain reaction reveals significant differences in allele distributions between Caucasians (Germany) and Africans (Gabon)

D. Kube, D. Schmidt, M. Mörmann, A.C. Uhlemann, J. Tomiuk, H. Tesch, P.G. Kremsner
microsatellites, interleukin-10 promoter, multiplex-PCR, Caucasian, African.

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