of skin products (protective creams, soaps, body lotions, shampoos, etc.).[11]

It has been suggested that sensitization to proteins from certain foods such as coconuts could be due to topical exposure through the skin. Whether it is through this route or the oral route by ingestion of foods containing products derived from coconuts, it is very probable that exposure to coconut proteins is now occurring from very early ages and thus the risk of sensitization to this fruit could facilitate an increase in allergic reactions to coconuts in the coming years.

In summary, we present a case of anaphylaxis in a child due to ingestion of coconut, and it is very probable that 7S globulin is the principal protein implicated in this allergic process.

## Ethical disclosures

**Protection of human and animal subjects.** The authors declare that no experiments were performed on humans or animals for this article.

**Data confidentiality.** The authors declare that no patient details appear in this article.

**Privacy rights and informed consent.** The authors declare that no patient details appear in this article.

## Conflicts of interest

We declare that we do not have any financial or personal relationship with regard to the submitted publication.

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http://dx.doi.org/10.1016/j.aller.2013.09.004

# Audit of the use of intravenous immunoglobulin for antibody deficiencies in a Clinical Immunology Unit

*To the Editor,*

Intravenous immunoglobulin preparations (IVIG) are indicated as the treatment of choice in a number of primary and secondary antibody deficiencies.[1–3] There are many options for IgG replacement, and the choice is an individual one based on many factors.[4] The present audit was developed to examine the current hospital practice of IVIG use for replacement therapy of antibody deficiencies in a Clinical Immunology Unit.

A two-month (February–March 2013) prospective audit of all IVIG use for replacement therapy of antibody deficiencies in two Immunology Day Hospitals was conducted at the Clinical Immunology Unit in the Gregorio Marañon Hospital in Madrid. Patients receiving subcutaneous immunoglobulin were not included in this study. Medical files and dispensing records were prospectively examined. A questionnaire was used. Solid organ transplantation recipients receiving IVIG therapy because of IgG hypogammaglobulinaemia and severe infections were not included in this audit as they were receiving replacement IVIG therapy in other facilities of the hospital.

Ninety-seven patients were included during the study period. The main four groups of distinct antibody deficiencies using IVIG at the time of the study were: Group 1: Common variable immunodeficiency (CVID, $n = 36$); Group 2: agammaglobulinaemic patients ($n = 3$, X-linked agammaglobulinaemia [$n = 2$], Good's Syndrome [$n = 1$]); Group 3: other primary antibody deficiencies ($n = 22$, including specific antibody deficiencies with normal IgG levels [$n = 12$], IgG subclass deficiency [$n = 6$], hyper IgM Syndrome [$n = 1$], other [$n = 3$]); and Group 4: secondary antibody deficiencies ($n = 36$, including IgG hypogammaglobulinaemia and recurrent infections secondary to lymphoma [$n = 14$], chronic lymphocytic leukaemia [$n = 2$], immunosuppressive therapy [$n = 9$], rituximab [$n = 2$], asthma or COPD using corticosteroids [$n = 5$] and other [$n = 4$]).

Mean age was 57, interval 19–87 years, 67 women, 30 men. Patients with secondary antibody deficiencies were significantly older ($69 \pm 14$ years) that CVID patients ($50 \pm 17$ years, ANOVA, $p < 0.01$) and agammaglobulinaemic patients ($44 \pm 17$ years, ANOVA, $p = 0.012$). Baseline mean serum IgG concentrations at the time of indication of IVIG replacement therapy (before commencement of IVIG) were as follows: Group 1, CVID: $349 \pm 166$ [47–570 mg/dL]; Group 2, agammaglobulinaemic patients: $208 \pm 25$ [180–230 mg/dL]; Group 3, specific antibody deficiencies with normal or high IgG levels: $1360 \pm 595$ mg/dL [706–2800 mg/dL], IgG subclass deficiencies with normal total IgG levels: $1084 \pm 380$ [762–1600 mg/dL]; and Group 4: secondary antibody deficiencies: $380 \pm 151$ [111–673 mg/dL].

Three preparations were available in the hospital at the time of the study: preparation A (5% concentration, $n = 45$, 46.4%), preparation B (10% concentration, $n = 38$, 39.2%) and preparation C (5% concentration, $n = 14$, 14.4%). The distribution of preparations in the four groups of patients was similar.

Overall, the protocol used followed current guidelines for IVIG replacement therapy including a dose of 200–800 mg/kg/month with an interval between doses of 3–4 weeks. Individual adjustments in the administered doses and intervals were performed taking into account the maintenance of IgG levels reached by each patient in combination with the clinical efficacy. The catabolic rate, tolerability of each product and logistic issues also affected the doses and interval between administrations of IVIG infusions.

Mean total prescription of IVIG was of 2695 g per month. Mean individual dose was of $28 \pm 12$ g/patient/month, interval 7–60 g/month (mean $465 \pm 195$ mg/kg/month [111–1110 mg/kg/month]).

Mean pre-infusion IgG serum concentration was $917 \pm 402$ mg/dL with an interval of 287–2720 mg/dL. Pre-infusion IgG levels obtained with distinct preparations were as follows: preparation A $873 \pm 413$ mg/dL, preparation B $885 \pm 289$ mg/dL and preparation C $1145 \pm 560$ mg/dL (ANOVA, $p = 0.07$). The ratio IgG/dose was as follows: preparation A $41 \pm 28$, preparation B $43 \pm 29$ and preparation C $69 \pm 44$ mg/dL/g infused of IVIG (ANOVA, $p = 0.012$).

CVID patients used similar doses of IVIG ($477 \pm 210$ mg/kg/month) to patients with secondary antibody deficiencies ($451 \pm 124$ mg/kg/month, ANOVA, $p = 0.59$) to maintain similar pre-infusion IgG levels ($766 \pm 239$ vs $814 \pm 276$ mg/dL, ANOVA, $p = 0.47$). Agammaglobulinaemic patients tended to require higher IVIG doses (656 mg/kg/month) than CVID and secondary hypogammaglobulinaemic patients (ANOVA, $p = 0.12$ and $p = 0.11$, respectively) to maintain similar IgG concentrations ($698 \pm 24$ mg/dL).

The mean infusion interval of time between IVIG doses was of 3.7 weeks. Distribution: 2 weeks: 13.5%, 3 weeks: 31.3%, 4 weeks: 42.7%.

Maximum IVIG infusion rate: 5% preparations: $159 \pm 50$ mL/h, interval 40–250 mL/h; 10% preparation: $133 \pm 69$ mL/h, interval 40–430 mL/h. Maximum IVIG infusion rates were significantly lower in those patients who referred to have adverse reactions ($128 \pm 57$ mL/h vs $158 \pm 58$ mL/h, Student's $t$ test, $p = 0.026$).

Mean total time of IVIG infusions was of $2.4 \pm 1.2$ h with an interval of 1–6.5 h. 20% of patients required more than 3 h to receive their IVIG doses.

Prevalence of adverse reactions: headache 19%, asthenia 4%, malaise 5%. No adverse reactions were reported by 72% of patients at the time of the study. Prevalence of patients with adverse reactions with the distinct preparations: preparation A 33%, preparation B 26%, preparation C 21% (Chi-square, $p = 0.63$). The prevalence of adverse reactions at the time of study was similar in the distinct indications (Chi-square, $p = 0.94$).

This audit emphasizes the variability in distinct parameters related with the administration of IVIG products for replacement therapy in clinical practice. Wide range of ages of the patients, heterogeneous conditions within different antibody deficiencies, patients requiring different doses according to their distinct IgG catabolic rates and higher pre-infusion IgG levels obtained with some preparates are also part of this variability. IVIG requires well-trained personnel to deal with this variability. On the other hand these characteristics should be taken into account when patients are scheduled in Day Hospitals and for the selection of potential candidates for switching to SCIG or IVIG-home-therapy. The availability of more than one preparation (with distinct concentrations) in the hospital offers an advantage to deal with this variability.

## Ethical disclosures

**Patients' data protection.** Confidentiality of data. The authors declare that they have followed the protocols of their work centre on the publication of patient data and that all the patients included in the study have received sufficient information and have given their informed consent in writing to participate in that study.

**Right to privacy and informed consent.** The authors have obtained the informed consent of the patients and/or subjects mentioned in the article. The author for correspondence is in possession of this document.

**Protection of human subjects and animals in research.** Protection of human and animal subjects. The authors declare that the procedures followed were in accordance with the regulations of the responsible Clinical Research Ethics Committee and in accordance with those of the World Medical Association and the Helsinki Declaration.

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http://dx.doi.org/10.1016/j.aller.2013.12.003

## Effects of cedar pollen extract on the immune system in vitro

*To the Editor,*

Allergic diseases affect a significant proportion of people around the world. The striking increase in the prevalence of allergic diseases over the last several decades involves various factors including changes in environmental pollutants, lifestyles, sanitary conditions, and diet.[1] Pollen is a factor for the development of Type 1 allergic diseases, including pollinosis – a common seasonal allergic disease characterised by symptoms such as rhinitis and conjunctivitis. Pollinosis that occurs during spring and autumn in Japan is related to the pollen from the trees of the Cypress family, including Japanese cedars. Most recently, cedar pollinosis has become prevalent in Japan not only among adults but also in children, and it has emerged as a major public health problem, due to its high prevalence and low natural recovery rate.[2]

Moreover, lines of epidemiological evidence indicate that exposure to pollen is also a risk factor for the development of allergic asthma. Although a grain of cedar pollen itself is too large to penetrate the lower airways, fine particles released from ruptured cedar pollen in contact with water are small enough to reach the lower airways and induce bronchial asthma.[3] In other studies, cedar pollen contaminated by air pollutants such as diesel exhaust particulates, yellow sand, and acid gases causes more persistent antigen-specific responses and airway inflammation.[4]

To date, five major and minor allergens (Cry j 1, Cry j 2, Cry j 3, CJP-4, and CJP-6) from Japanese cedar pollen have been identified and characterised.[5] Several studies have reported that specific IgE antibodies to Cry j 1 and Cry j 2 are detected in up to 90% of the patients suffering from cedar pollinosis, and both allergens are recognised as major potential allergens for cedar pollinosis.[5] Thus Cry j 1 and Cry j 2 are considered to be responsible for allergic airway inflammation through adaptive immune responses such as inducing specific IgE. However, the underlying pathological mechanisms at cellular and molecular levels remain to be elucidated, whereas other allergens such as house dust mite allergens, an indoor inhaled allergen, have been well studied both in vitro and in vivo.[6,7]

Antigen-presenting cells (APCs), including dendritic cells (DCs) and macrophages, play important roles in the immune system. APCs in the airways take up antigens and migrate to lymphoid organs such as local lymph nodes, where they present antigen-derived peptides on their MHC molecules after maturation. There, antigen-specific T cells differentiate into effector T cells via an interaction with APCs. Therefore, APCs and lymphocytes play crucial roles in the immune system and in the mechanisms of allergic airway inflammation.

To elucidate the precise cellular and molecular events that are induced by Cry j 1 and Cry j 2 contained in cedar pollen extract, we investigated the effects of cedar pollen extract on the responses of bone marrow-derived cells and splenocytes from atopic prone NC/NgaTendCrlj male mice (Charles River Japan, Osaka, Japan) in vitro, and we determined which cells and biomarkers are more appropriate and useful for evaluations of the allergic effects of cedar pollen. The procedures for all animal studies were approved by the Animal Research Committee at Kyoto University.

Bone marrow cells were differentiated using a modified protocol of Lutz et al.[8] In brief, they were cultured in R10 medium containing 20 ng/mL GM-CSF (Sigma, St. Louis, MO, USA). The bone marrow-derived cells were exposed to cedar pollen extract (Cosmo Bio Co., Tokyo, Japan) at 0, 1, or 5 μg/mL as the dose of Cry j 1 + Cry j 2 for 24 h. The DEC205, CD86, and CD14 protein expressions on the cell surface were evaluated by fluorescence-activated cell sorting (FACS), using Ab for CD86, CD14 (BD Biosciences PharMingen, San Diego, CA, USA) and DEC205 (Milteny Biotec, Gladbach, Germany). DEC205 is an endocytic receptor and CD86 is critical for T lymphocyte activation and differentiation of T helper subsets. CD14 binds to a variety of microbial products. Splenocytes were exposed to cedar pollen extract at the same dose for 72 h. The release of IL-2 and IL-4 in the culture supernatants was evaluated by enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, USA).

It is well established that APCs are crucial to the immune system. They do not only activate naïve T cells but also regulate T cell responses by various patterns of co-stimulatory signals and cytokine expression. We found that the cedar pollen extract significantly increased the percentages of DEC205, CD86, and CD14 expression on bone