

LETTERS TO THE EDITOR

## Soluble CD40 ligand in asthma patients during allergen challenge

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CD40–CD40 ligand (CD40L) interaction is necessary for the development of antigen-specific immune responses. CD40L provides the signal required for the differentiation of B cells, and is crucial for immunoglobulin isotype switching from IgM to IgE or IgG synthesis [1]. In an animal model of allergic asthma, production of allergen-specific IgE was suppressed in both CD40<sup>−/−</sup> and CD40L<sup>−/−</sup> strains, pulmonary inflammation was not affected in either strain, and bronchial hyperreactivity was attenuated only in CD40L<sup>−/−</sup> mice [1]. CD40L is present within the circulation also in soluble form (sCD40L). *In vitro*, many functions of membrane-bound CD40L can be substituted by sCD40L [2,3]. It is not known whether allergen challenge in humans results in increased expression of CD40L, and what is the predominant source of CD40L. We therefore evaluated sCD40L levels in plasma of asthma patients during allergen challenge in relation to lung function and levels of T-cell and platelet activation markers [4,5].

Thirty-six asthma patients (20 male, 16 female; mean age  $23.7 \pm 4.6$  years) had confirmed IgE-mediated hypersensitivity to one of the major components of the house dust mite *Dermatophagoides pteronyssinus* (Dp). The patients had mild asthma [mean forced expiratory volume in 1 s (FEV<sub>1</sub>) =  $85.5 \pm 6.9\%$  predicted], and before the initial visit did not receive allergen-specific immunotherapy or any antiasthma medication, except for sporadic application of inhaled short-acting  $\beta_2$ -agonists.  $\beta_2$ -Agonists were withheld for at least 2 weeks before the study. Twenty-six healthy subjects (16 male and 10 female; mean age  $22.7 \pm 3.5$  years; mean FEV<sub>1</sub> =  $98.8 \pm 7.9\%$  predicted) without IgE-mediated hypersensitivity to common aeroallergens were used as

controls (HCs). The study was approved by the local ethics committee. Nonspecific bronchial reactivity and Dp-specific bronchial reactivity were evaluated by histamine and Dp bronchial challenges, respectively [4,5]. Nonspecific bronchial reactivity was expressed as the histamine concentration causing a 20% fall in FEV<sub>1</sub> (PC<sub>20</sub>). Specific bronchial reactivity was expressed as the Dp dose causing a 20% reduction in FEV<sub>1</sub> (PD<sub>20</sub>). Healthy controls were sham challenged with 0.9% NaCl. Blood samples were collected before bronchial allergen challenge ( $T_0$ ), at 30–45 min ( $T_{EAR}$ ), at 6–8 h ( $T_{LAR}$ ) and at 24 h ( $T_{24}$ ) after administration of the last allergen dose [5]. Serum total IgE (tIgE) and anti-Dp-specific IgE (DpIgE) were evaluated using the UniCap system (Pharmacia, Sweden). Serum sCD25, plasma sP-selectin and plasma sCD40L levels were evaluated by enzyme-linked immunosorbent assay (R&D Systems). All results are presented as means with standard deviations ( $\bar{x} \pm SD$ ). Continuous variables were compared using the repeated measures ANOVA test with *post hoc* analysis. IgE concentrations, PC<sub>20</sub> values and PD<sub>20</sub> values were logarithmically transformed. Pearson linear correlation was used to estimate associations between studied parameters. The area under the curve (AUC) of plasma concentrations of individual studied markers plotted vs. time was calculated for each patient, and the AUC values were used for analysis.

Before allergen challenge, the mean plasma sCD40L level in asthma patients ( $2.97 \pm 0.185$  ng mL<sup>−1</sup>) was greater than that in HCs ( $2.05 \pm 1.17$  ng mL<sup>−1</sup>;  $P = 0.0001$ ) (Fig. 1A). Similarly, mean plasma sP-selectin levels ( $41 \pm$  ng mL<sup>−1</sup> vs.  $30 \pm 7$  ng mL<sup>−1</sup>,  $P < 0.0001$ ) and mean serum sCD25 levels ( $743 \pm 237$  pg mL<sup>−1</sup> vs.  $454 \pm 103$  pg mL<sup>−1</sup>;  $P < 0.0001$ ) were significantly greater in asthma patients than in HCs (Fig. 1B,C). Significantly increased plasma sCD40L (mean AUC =  $109.9 \pm 13$ ) and plasma sP-selectin (mean AUC =  $1224 \pm 254$ ) concentrations were seen in asthma patients in comparison with HCs (mean AUC =  $54.4 \pm 15$ ,  $P < 0.001$ , and mean AUC =  $752 \pm 199$ ,  $P < 0.001$ , for sCD40L and sP-selectin respectively) over the course of a 24-h period. A similarly significantly increased serum sCD25 concentration

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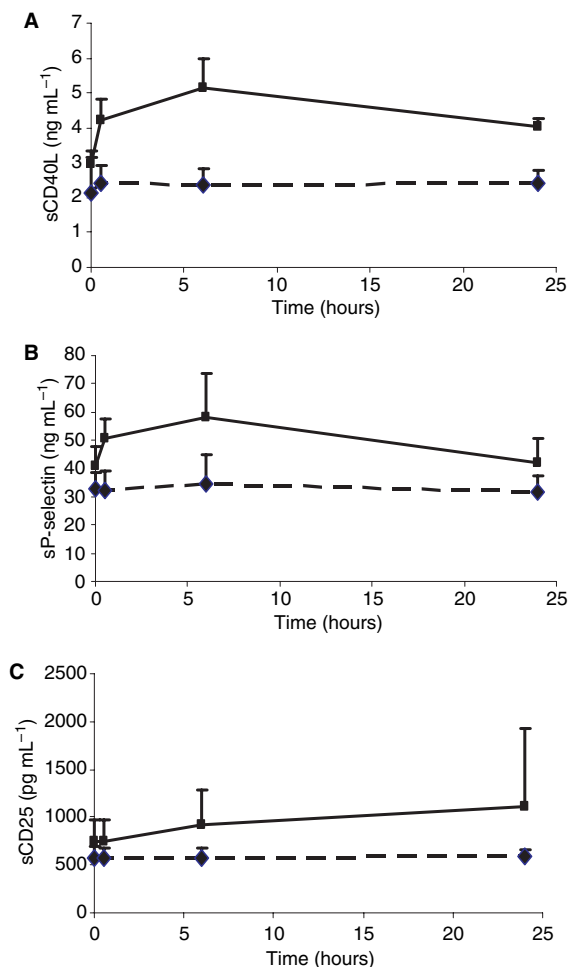


**Fig. 1.** Soluble CD40 ligand in asthma patients during allergen challenge. Changes in plasma sCD40L levels (A), plasma sP-selectin levels (B) and serum sCD25 levels (C) during allergen challenge. The lines represent mean values, and error bars represent standard deviations. ♦, sham controls; ■, allergen challenge.

was seen in asthma patients in comparison with HCs (mean AUC = 23 339 ± 11 838 vs. mean AUC = 13 690 ± 2724, for asthma patients and HCs respectively,  $P = 0.001$ ). The mean levels of sCD40L and sP-selectin reached the greatest values at  $T_{LAR}$ , whereas the greatest mean serum concentration of sCD25 was detected at  $T_{24}$  (Fig. 1). Baseline level of sCD40L correlated with log DpIgE ( $r = 0.3577$ ,  $P = 0.0322$ ) and with sP-selectin level ( $r = 0.449$ ,  $P = 0.006$ ) but not with log PC<sub>20</sub>, log PD<sub>20</sub>, log tIgE or baseline sCD25 level. Similarly, logDpIgE correlated with the AUC of sCD40L ( $r = 0.774$ ,  $P < 0.0001$ ) and the AUC of sP-selectin ( $r = 0.662$ ,  $P < 0.0001$ ) during the challenge, but not with the AUC of sCD25. We were not able to demonstrate any association between plasma sCD40L level and bronchial reactivity to histamine (data not shown).

Our results indicate that strong upregulation of sCD40L in allergic asthma patients during allergen challenge is associated with an IgE-dependent immediate allergic reaction and may

contribute to a positive feedback loop leading to upregulation of allergen-specific IgE synthesis after allergen challenge. The mean concentration of sCD40L at  $T_{LAR}$  was similar or even greater than that seen in cardiovascular or metabolic diseases such as metabolic syndrome, indicating that CD40L could be involved in the pathogenesis of the asthmatic response to allergen challenge [6]. Moreover, the rise in plasma sCD40L concentration reflects increased cellular CD40L expression, so local activation of CD40 by CD40L may be much greater than that calculated solely on the basis of mean plasma concentration. Platelets are the predominant source of intravascular sCD40L, and therefore both platelets and CD40L should be considered interesting therapeutic targets in allergic asthma patients. Our results are consistent with other studies demonstrating that platelets are the predominant source of intravascular sCD40L in several pathologic conditions in humans [7,8]. The plasma kinetics of sCD40L is similar to that of sP-selectin, a marker that, in allergic asthma patients, predominantly derives from platelets [5]. The correlation between baseline plasma sCD40L level and DpIgE concentration, but not with any other asthma parameter, indicates a link between the allergen-specific immune response and increased CD40L production. Upregulation of CD40L after exposure to allergen may play an important role in amplification of IgE-mediated inflammation induced by allergen exposure, possibly by further increasing allergen-specific IgE synthesis. However, it cannot be determined from our study whether the function of CD40L is mediated by a membrane form expressed on the platelet surface or by a soluble form.

## Disclosure of Conflict of Interests

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## Estimation of a potentially preventable fraction of venous thromboembolism: a community-based prospective study

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Venous thromboembolism (VTE), encompassing deep-vein thrombosis (DVT) and pulmonary embolism (PE), is frequent and associated with substantial morbidity and mortality [1–3].

The reduction of sudden deaths related to PE or death following undiagnosed and untreated VTE could only be achieved through efficacious prophylaxis. Moreover, a successful preventive strategy will reduce symptomatic VTE and thereby morbidity related to curative anticoagulant treatment and post-thrombosis syndrome. Recommendations for VTE prophylaxis in identifiable clinical situations have been published [4]. Estimating the proportion of symptomatic VTE that is related to easily identifiable situations, such as hospitalization and/or diagnosis of cancer, is of interest. A low proportion would tell us that any efficacious prevention would have only a minor impact on overall VTE occurrence as so-called idiopathic VTE occurring like a storm in a blue sky can hardly be prevented. Conversely, a high proportion would raise questions regarding the effectiveness of prophylactic recommendations. As part of two-step reasoning, this study focused

on estimating the incidence of hospital and/or cancer-related VTE.

All physicians in Brest, France, involved in the diagnosis of VTE who agreed to join the epidemiological group called EPI-GETBO [1] were once again contacted. This group consisted of radiologists, nuclear physicians and angiologists. Hospital care is provided by Brest University Hospital and Clermont-Tonnerre Military Public Hospital. Angiologists use compression ultrasonography (CUS) and radiologists perform mainly helicoidal computed tomography. Both provide diagnostic facilities for mainly outpatients but also patients hospitalized in private hospitals.

A standardized data form was completed for every patient with a proven acute DVT or PE during a 1-year period. The following diagnostic tests were used: helicoidal computed tomography, ventilation-perfusion lung scan and venous CUS. VTE was diagnosed as previously defined [1]. DVT of the lower limbs, either distal or proximal (involving the popliteal vein or above), had to be confirmed by real time B mode CUS. The diagnostic criteria for PE were: (i) a high probability lung scan, (ii) a proximal DVT shown by CUS, or (iii) a positive helicoidal computed tomography. The data form focused on the clinical setting at the time of the diagnosis (home, acute-care hospital, nursing home) and requested information on hospitalization in the previous 3 months and history of cancer. Cancer was defined as a diagnosis of cancer other than basal-cell or squamous-cell carcinoma of the skin within 6 months before VTE diagnosis, any treatment for cancer within the previous 6 months, or recurrent or metastatic cancer. It contained also the following information: date of diagnosis, first letter of name, surname, sex and year of birth, patient's address, previous episode of VTE, type and result of the diagnostic test. All the data were sent to one of us (E.O.) and entered into a

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