

EDITORIAL

Cytotoxic T lymphocyte antigen 4 polymorphisms and allergic asthma

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Introduction

Allergic asthma is a complex disease characterized by reversible airway obstruction, elevated levels of IgE, chronic eosinophilic airway inflammation, lung tissue remodelling, and airway hyper-reactivity (AHR) to bronchospasmogenic stimuli. T helper type-2 (Th2) lymphocytes, which produce a distinctive set of cytokines, including IL4, IL5, IL9, IL10, and IL13, play a crucial role in the initiation and progression of allergic asthma. Therefore, knowledge of the activation requirements of T cells and the subsequent differentiation into Th1, Th2, or regulatory T cell subsets is important to understand the aberrant Th2-mediated inflammatory responses to allergens observed in allergic individuals, including those with asthma. Moreover, this knowledge can be used to design novel therapeutic strategies that target the Th2-lymphocyte. An area of research that has continued to attract a lot of attention is the role of the CD28/CTLA4:B7-1/B7-2 co-stimulatory pathway that is crucial for the activation of naïve T cells (Fig. 1). Interestingly, several studies have shown linkage of total serum IgE or other allergy phenotypes to chromosome 2q32–q33, a region encoding both CD28 and CTLA4 genes. In this issue of *Clinical and Experimental Allergy*, Yang et al. [1] report that a polymorphism in CTLA4 is associated in females, but not in males, to elevated serum levels of total IgE and allergic rhinitis.

T cell co-stimulation

The current concept of T lymphocyte activation is originally derived from the two-signal model of B cell activation, first proposed by Bretscher and Cohn in 1970 [2]. Analogous to this model, T cell activation requires two independent signals, one through the T cell receptor (TCR) provided by engagement of the peptide-MHC class II complex on antigen-presenting cells (APCs) and a second so-called co-stimulatory signal. Various receptor–ligand pairs for T cell co-stimulation have been identified; however, the CD28/CTLA4:B7-1/B7-2 pathway is crucial for the primary activation of naïve T cells (Fig. 1). CD28 is constitutively present on the cell-surface of T cells and has two ligands, B7-1 (CD80) and B7-2 (CD86), which are expressed by APCs. On most APC populations, B7-2 is expressed constitutively at low levels and is rapidly upregulated upon APC activation, whereas B7-1 is inducibly expressed later after activation. CD28 signals promote T cell activation by augmenting and sustaining T cell responses initiated by TCR signalling. Blockade of CD28 during activation of naïve T cells renders these cells anergic to secondary activation, even when restimulated in the presence of CD28 ligation [3]. A second receptor for the B7 ligands,

cytotoxic T lymphocyte antigen 4 (CTLA4, CD152), has been identified, which has a higher affinity for B7-1 molecules than CD28 [4]. In contrast to CD28, CTLA4 is expressed only on activated T cells and is a powerful negative regulator of T cell activation. The importance of negative regulatory signals through CTLA4 is illustrated in mice deficient in CTLA4. These mice manifest massive lymphoproliferative disease, which is lethal by 3 weeks of age. Thus, CD28 and CTLA4 compete for the same ligands, B7-1 and B7-2, but have counter-regulatory effects on T cell activation.

Role of CD28/CTLA4 co-stimulatory pathways in allergic asthma

The importance of the CD28/CTLA4:B7-1/B7-2 co-stimulatory pathway in allergic asthma has been studied *in vitro* using peripheral blood mononuclear cells (PBMC), bronchoalveolar lavage (BAL) T cells, and bronchial tissue explants. Larche et al. [5] demonstrated that, in contrast to peripheral blood CD4⁺ T cell lines, allergen-induced proliferation and IL5 production of PBMC were inhibited by anti-B7-2 but not anti-B7-1. Moreover, allergen-induced proliferation of BAL T cells was also inhibited by anti-B7-2. Similarly, Van Neerven et al. [6] demonstrated that allergen- and antigen-specific T cells of allergic patients and non-allergic control persons are equally dependent on co-stimulation via the CD28:B7-2 pathway for their proliferation and cytokine mRNA production. Blockade of both B7-1 and B7-2 by CTLA4-Ig, a soluble form of CTLA4, appeared to be slightly more effective than blocking B7-2 alone.

The group of Holgate has studied the role of CD28 co-stimulation using the *ex vivo* response of bronchial explants to allergens. Blockade of either B7-1 or B7-2 inhibited allergen-induced IL5 production by bronchial tissue explants from mild atopic asthmatic subjects [7], but not by those of subjects with moderately severe asthma [8]. In contrast, blocking of CD28 co-stimulation effectively inhibited allergen-induced IL5 production in PBMC cultures from the same subjects. The difference in the requirement of B7-1 and B7-2 for allergen-induced activation of T cells may reflect differences in cell-surface expression of these molecules on the local APCs involved. Compared with peripheral blood, in airway tissue a different (sub)type(s) of APC may present allergen to T cells or B7-1 may be higher expressed due to the ongoing local inflammatory process.

Mouse models of experimental asthma

To delineate the role of the CD28/CTLA4:B7-1/B7-2 co-stimulatory pathways both *in vivo* and *in vitro*, the mouse has

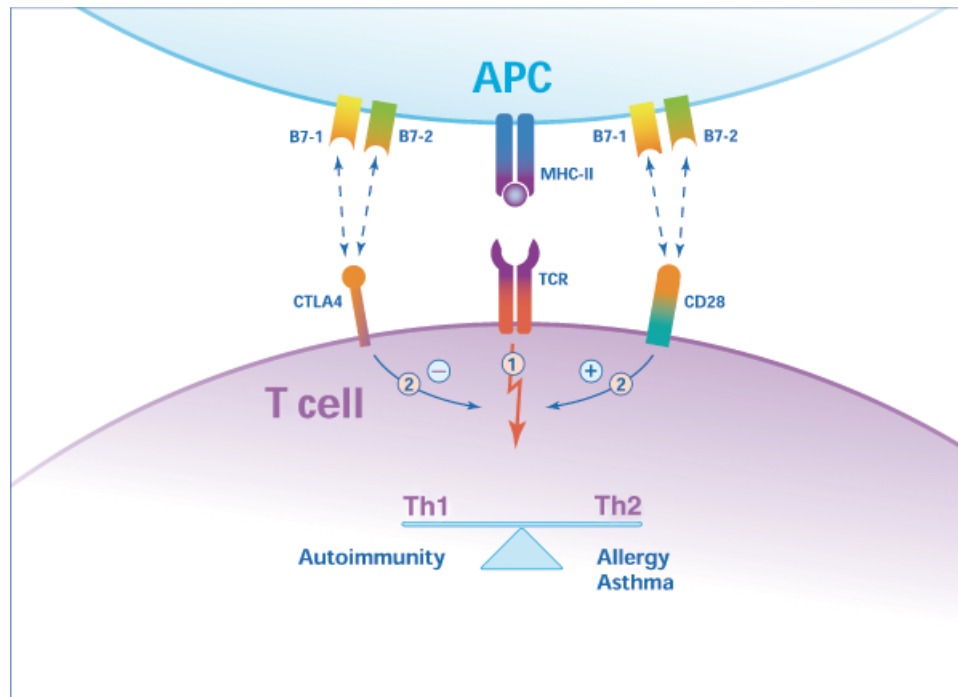


Fig. 1. Schematic representation of the two-signal model of T cell activation. Triggering of the T cell receptor (TCR) by the peptide-MHC class II complex provides signal one ①. The co-stimulatory signals ② are delivered by ligation of CTLA4 and CD28 on the T cell by B7-1 and B7-2 on antigen-presenting cells (APC). CD28 and CTLA4 have counter-regulatory effects on T cell activation and favour the differentiation of naïve T cells into Th2 or Th1, respectively. Th1 and Th2 cells play an important role in the development of, respectively, organ-specific autoimmunity and allergic diseases, including asthma.

been used extensively. First, CD28 is critical for the differentiation of naïve T cells into Th2 cells, whereas CTLA4 limits Th2-differentiation [9–11]. Using T cells from CTLA4-deficient mice, differentiation of Th2 cells becomes even independent of the Th2-skewing cytokine IL4 [12]. Thus, strong T cell activation provided by TCR and CD28 signals favours Th2-generation, whereas weak CD28 co-stimulatory signals lead to Th1 differentiation [12].

Second, the critical role of CD28 co-stimulation in the development of antigen-induced airway manifestations of asthma has been demonstrated in blocking and gene targeting studies in mouse models *in vivo*. It has been shown that blockade of B7-1 and B7-2 during antigen sensitization or challenge strongly inhibits serum IgE levels and antigen-induced AHR and eosinophilic airway inflammation [13, 14]. In all these studies, a decrease in Th2-cytokine production was observed in either the BAL fluid or in T cell recall cultures. In agreement herewith, no asthma manifestations could be induced in CD28-deficient mice [15], B7-1/B7-2-double-deficient mice [16], or CD28/CTLA4-double-deficient mice [17]. Altogether these data firmly demonstrate the crucial role of CD28 co-stimulation in the development of allergic sensitization and antigen-induced asthma manifestations, suggesting that the inhibition of CD28:B7 co-stimulation pathway may be an interesting therapeutic strategy.

However, the clinical situation is more complex since disease is already established in asthmatic subjects and memory and effector T cells are less dependent on CD28 co-stimulation [18]. Interestingly, in a mouse model of established antigen-induced asthma manifestations, CTLA4-Ig was shown to significantly suppress both serum IgE levels

and airway eosinophilia, but not hyper-reactivity [19]. These suppressive effects were more pronounced in a 'mild' compared with a more 'severe' model of experimental asthma. This is in agreement with the observation that T cells from mild, but not moderately severe, asthma subjects are sensitive to blockade of CD28 co-stimulation [8]. The beneficial effects of therapeutic inhibition of CD28 co-stimulation in asthmatic subjects may therefore be limited to mild forms of asthma.

CTLA4 polymorphisms and asthma

Genome-wide screens for allergic asthma in different populations have provided several promising linkages, including a locus on chromosome 2q32–q33 that contains the genes for CD28 and CTLA4 [20]. Linkage of serum IgE levels or other allergy phenotypes to this locus has been demonstrated in different populations [21–25]. Considering their important role in T cell activation and differentiation, CD28 and CTLA4 were classified as candidate genes and analysed in subsequent association studies. None of these studies has demonstrated a significant association of CD28 polymorphisms with asthma or atopy [26–28]. In contrast, some studies have shown a significant association of different CTLA4 polymorphisms (Fig. 2) with asthma or atopy [27, 29, 30], whereas other studies did not find an association [26, 28]. In this issue, Yang et al. [1] report that a polymorphism in CTLA4 (+49A/G) is associated in females, but not in males, to elevated serum levels of total IgE. Previous studies have

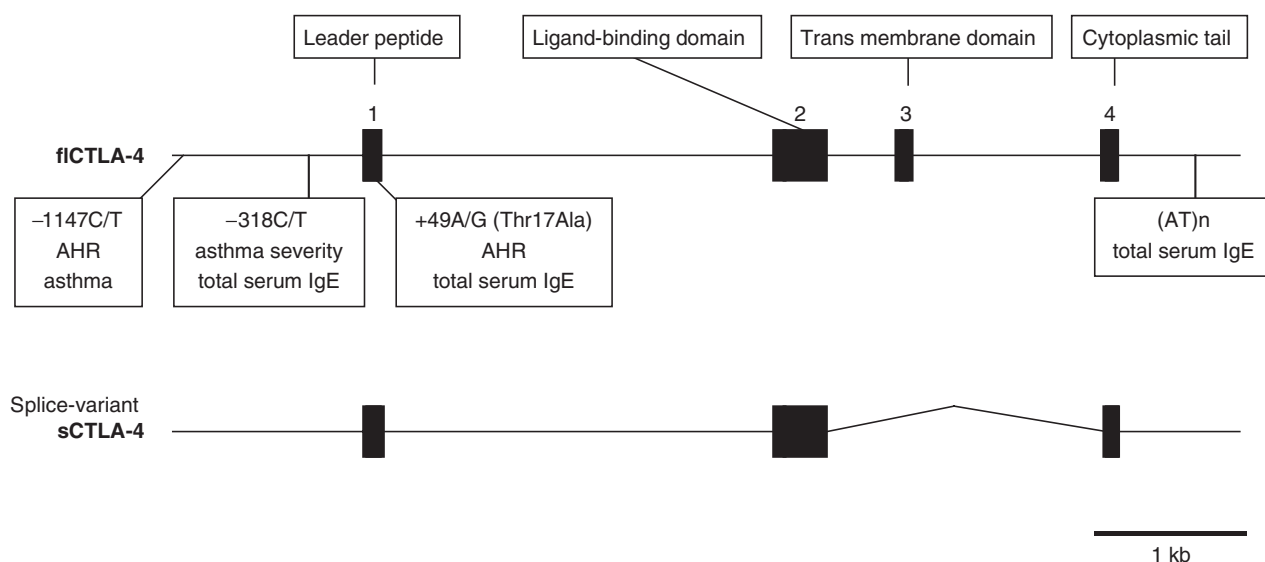


Fig. 2. Polymorphisms in human CTLA4. CTLA4 consists of four exons, indicated by black boxes. Introns are represented by thin lines between the exons. Differential splicing of exon 3 is indicated by tilted lines. flCTLA4, full-length CTLA4; sCTLA4, soluble CTLA4 lacking the transmembrane domain. Reported associations with asthma, airway hyper-reactivity (AHR), and total serum IgE are indicated (for details and references, see text).

not reported such a gender difference [26, 27, 29], but differences in the set-up and the size of these studies make it possible that such an effect, if present, would not have been observed. Therefore, replication of this important finding in independent studies is clearly warranted. The mechanism by which this CTLA4 (+49A/G) polymorphism may lead to increased serum levels of total IgE can only be speculated at. At the protein level, the polymorphism results in a conserved amino acid substitution (17Thr/Ala), which may well be without functional consequences. Furthermore, strong linkage disequilibrium between different CTLA4 polymorphisms makes it quite possible that the causative mutation is located at some distance from the +49A/G polymorphism. If this mutation leads to a dysfunction of negative signalling through CTLA4, an increased susceptibility to generate Th2-responses may be the consequence, as discussed above. In agreement herewith, it has been shown that blockade of CTLA4 at the time of antigen sensitization favoured Th2-generation and antigen-specific IgE production in BALB/c mice resulting in elevated airway manifestations of asthma [31]. However, CTLA4 may influence Th2-dependent total serum IgE levels by completely different mechanisms. First, triggering of CTLA4 on regulatory T cells, which constitutively express CTLA4, can enhance the suppressor function of these cells [32, 33]. Second, CTLA4 can inhibit T cell proliferation indirectly by enhancing the levels of indoleamine 2,3-dihydrogenase in APCs [34].

Howard et al. [27] also observed significant association of the CTLA4 – 1147C/T polymorphism with AHR and asthma, but not for allergy-related phenotypes. Subjects with risk genotypes for both AHR (– 1147CT or TT) and elevated IgE levels (+49AA) were 4.5 times more likely to have asthma than subjects with both non-risk genotypes. The authors suggested that the biological implication of the CTLA4 – 1147C/T polymorphism, which lies within the consensus-binding site for transcription factors, may be the level of CTLA4 mRNA expression [27].

Recently, several common organ-specific autoimmune diseases have been shown to be linked to CTLA4 polymorphisms [35]. In this study, autoimmune-protective and susceptible haplotypes were identified. The CTLA4 polymorphism +49A/G appeared to be part of the autoimmune-protective haplotype (+49A-CT60A). It is noteworthy that the +49A/G mutation could be excluded as the causative mutation, which, according to this study, is most likely located close, or perhaps identical to the CT60A/G polymorphism, which is located a few kb downstream of the CTLA4 gene. The CT60 polymorphism has not been determined in any of the studies involving allergy and asthma, making it difficult to draw conclusions about the involvement of CT60-polymorphism in the susceptibility for allergic diseases. However, the CTLA4 autoimmune-protective haplotype (+49A-CT60A) appears to be associated with increased susceptibility to IgE production and allergic disease [1, 27]. Ueda et al. [35] also examined the consequence of the autoimmune-protective and -susceptible (+49G-CT60G) haplotypes for the mRNA expression levels of two known CTLA4 isoforms, full-length CTLA4 (flCTLA4) and soluble CTLA4 (sCTLA4). The ratio of sCTLA4 to flCTLA4 mRNA splice forms in unstimulated CD4 T cells was 50% lower in individuals with the autoimmune-susceptible haplotype compared with those with the protective haplotype. sCTLA4 is present in human serum and has been shown to bind B7-1 [36]. Serum levels of sCTLA4 may therefore play a role in the susceptibility to autoimmune diseases and probably also in the development of allergy and asthma. Currently, nothing is known about the serum levels of sCTLA4 in allergy and asthma subjects.

Conclusions

Genetic susceptibility to atopy and asthma appears to be due to multiple genes that interact with each other and the

environment. A genetic association of the +49A/G polymorphism in the immune-braking gene CTLA4 with serum total IgE levels in atopic and asthmatic subjects is demonstrated in at least some (sub)populations. The precise mechanism of how the different haplotypes affect increased serum IgE levels and other asthma manifestations can only be speculated at, but differential splicing of CTLA4, resulting in competition of different CTLA4 isoforms, may play a role. It appears likely that the increased serum IgE concentration is the result of enhanced T cell activation leading to increased generation of Th2 cells. The promotion of Th2-skewing may be mediated by (i) decreased negative signalling through cell-surface CTLA4, (ii) decreased function of regulatory T cells, (iii) decreased indoleamine 2,3-dihydrogenase activity of APCs, and (iv) increased serum levels of sCTLA4. Future studies on the functional implications of CTLA4 polymorphisms may reveal which of these mechanisms, if any, is implicated.

References

- Yang KD, Liu C-A, Chang J-C et al. Polymorphism of the immune-braking gene CTLA-4 (+49) involved in gender discrepancy of serum total IgE levels and allergic disease. *Clin Exp Allergy* 2004; 34:32–7.
- Bretscher P, Cohn M. A theory of self-nonsel discrimination. *Science* 1970; 169:1042–9.
- Schwartz RH. Costimulation of T lymphocytes: the role of CD28, CTLA-4, and B7/BB1 in interleukin-2 production and immunotherapy. *Cell* 1992; 71:1065–8.
- Collins AV, Brodie DW, Gilbert RJ et al. The interaction properties of costimulatory molecules revisited. *Immunity* 2002; 17:201–10.
- Larche M, Till SJ, Haselden BM et al. Costimulation through CD86 is involved in airway antigen-presenting cell and T cell responses to allergen in atopic asthmatics. *J Immunol* 1998; 161:6375–82.
- Van Neerven RJ, Van de Pol MM, Van der Zee JS, Stiekema FE, De Boer M, Kapsenberg ML. Requirement of CD28–CD86 costimulation for allergen-specific T cell proliferation and cytokine expression. *Clin Exp Allergy* 1998; 28:808–16.
- Jaffar ZH, Stanciu L, Pandit A, Lordan J, Holgate ST, Roberts K. Essential role for both CD80 and CD86 costimulation, but not CD40 interactions, in allergen-induced Th2 cytokine production from asthmatic bronchial tissue: role for alphabeta, but not gammadelta, T cells. *J Immunol* 1999; 163:6283–91.
- Lordan JL, Davies DE, Wilson SJ et al. The role of CD28–B7 costimulation in allergen-induced cytokine release by bronchial mucosa from patients with moderately severe asthma. *J Allergy Clin Immunol* 2001; 108:976–81.
- Rulifson IC, Sperling AI, Fields PE, Fitch FW, Bluestone JA. CD28 costimulation promotes the production of Th2 cytokines. *J Immunol* 1997; 158:658–65.
- Oosterwegel MA, Mandelbrot DA, Boyd SD et al. The role of CTLA-4 in regulating Th2 differentiation. *J Immunol* 1999; 163:2634–9.
- Kato T, Nariuchi H. Polarization of naïve CD4⁺ T cells toward the Th1 subset by CTLA-4 costimulation. *J Immunol* 2000; 164:3554–62.
- Bour-Jordan H, Grogan JL, Tang Q, Auger JA, Locksley RM, Bluestone JA. CTLA-4 regulates the requirement for cytokine-induced signals in T(H)2 lineage commitment. *Nat Immunol* 2003; 4:182–8.
- Krinzman SJ, DeSanctis GT, Cernadas M et al. Inhibition of T cell costimulation abrogates airway hyperresponsiveness in a murine model. *J Clin Invest* 1996; 98:2693–9.
- Van Oosterhout AJ, Hofstra CL, Shields R et al. Murine CTLA4-IgG treatment inhibits airway eosinophilia and hyperresponsiveness and attenuates IgE upregulation in a murine model of allergic asthma. *Am J Respir Cell Mol Biol* 1997; 17:386–92.
- Mathur M, Herrmann K, Qin Y et al. CD28 interactions with either CD80 or CD86 are sufficient to induce allergic airway inflammation in mice. *Am J Respir Cell Mol Biol* 1999; 21:498–509.
- Mark DA, Donovan CE, De Sanctis GT et al. B7-1 (CD80) and B7-2 (CD86) have complementary roles in mediating allergic pulmonary inflammation and airway hyperresponsiveness. *Am J Respir Cell Mol Biol* 2000; 22:265–71.
- Deurloo DT, van Berkel MA, van Esch BC et al. CD28/CTLA4 double deficient mice demonstrate crucial role for B7 costimulation in the induction of allergic lower airways disease. *Clin Exp Allergy* 2003; 33:1297–304.
- London CA, Lodge MP, Abbas AK. Functional responses and costimulator dependence of memory CD4⁺ T cells. *J Immunol* 2000; 164:265–72.
- Deurloo DT, van Esch BC, Hofstra CL, Nijkamp FP, van Oosterhout AJ. CTLA4-IgG reverses asthma manifestations in a mild but not in a more 'severe' ongoing murine model. *Am J Respir Cell Mol Biol* 2001; 25:751–60.
- Steinke JW, Borish L, Rosenwasser LJ. Genetics of hypersensitivity. *J Allergy Clin Immunol* 2003; 111:S495–501.
- Xu J, Postma DS, Howard TD et al. Major genes regulating total serum immunoglobulin E levels in families with asthma. *Am J Hum Genet* 2000; 67:1163–73.
- Koppelman GH, Stine OC, Xu J et al. Genome-wide search for atopy susceptibility genes in Dutch families with asthma. *J Allergy Clin Immunol* 2002; 109:498–506.
- Wjst M, Fischer G, Immervoll T et al. A genome-wide search for linkage to asthma. German Asthma Genetics Group. *Genomics* 1999; 58:1–8.
- Ober C, Tsalenko A, Parry R, Cox NJ. A second-generation genomewide screen for asthma-susceptibility alleles in a founder population. *Am J Hum Genet* 2000; 67:1154–62.
- Dizier MH, Besse-Schmittler C, Guilloud-Bataille M et al. Genome screen for asthma and related phenotypes in the French EGEA study. *Am J Respir Crit Care Med* 2000; 162:1812–8.
- Nakao F, Ihara K, Ahmed S et al. Lack of association between CD28/CTLA-4 gene polymorphisms and atopic asthma in the Japanese population. *Exp Clin Immunogenet* 2000; 17:179–84.
- Howard TD, Postma DS, Koppelman GH et al. Fine mapping of an IgE-controlling gene on chromosome 2q: Analysis of CTLA4 and CD28. *J Allergy Clin Immunol* 2002; 110:743–51.
- Heinzmann A, Plesnar C, Kuehr J, Forster J, Deichmann KA. Common polymorphisms in the CTLA-4 and CD28 genes at 2q33 are not associated with asthma or atopy. *Eur J Immunogenet* 2000; 27:57–61.
- Lee SY, Lee YH, Shin C et al. Association of asthma severity and bronchial hyperresponsiveness with a polymorphism in the cytotoxic T-lymphocyte antigen-4 gene. *Chest* 2002; 122:171–6.
- Hizawa N, Yamaguchi E, Jinushi E, Konno S, Kawakami Y, Nishimura M. Increased total serum IgE levels in patients with asthma and promoter polymorphisms at CTLA4 and FCER1B. *J Allergy Clin Immunol* 2001; 108:74–9.
- Hellings P, Vandenberghe P, Kasran A et al. Blockade of CTLA-4 enhances allergic sensitization and eosinophilic airway inflammation in genetically predisposed mice. *Eur J Immunol* 2002; 32:585–94.
- Takahashi T, Tagami T, Yamazaki S et al. Immunologic self-tolerance maintained by CD25(+)CD4(+) regulatory T cells

- constitutively expressing cytotoxic T lymphocyte-associated antigen 4. *J Exp Med* 2000; 192:303–10.
- 33 Read S, Malmstrom V, Powrie F. Cytotoxic T lymphocyte-associated antigen 4 plays an essential role in the function of CD25(+)CD4(+) regulatory cells that control intestinal inflammation. *J Exp Med* 2000; 192:295–302.
- 34 Grohmann U, Orabona C, Fallarino F et al. CTLA-4-Ig regulates tryptophan catabolism *in vivo*. *Nat Immunol* 2002; 3:1097–101.
- 35 Ueda H, Howson JM, Esposito L et al. Association of the T-cell regulatory gene CTLA4 with susceptibility to autoimmune disease. *Nature* 2003; 423:506–11.
- 36 Oaks MK, Hallett KM. Cutting edge: a soluble form of CTLA-4 in patients with autoimmune thyroid disease. *J Immunol* 2000; 164:5015–8.

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