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# Thromboxane $A_2$ modulates interaction of dendritic cells and T cells and regulates acquired immunity

Kenji Kabashima[1,2,8], Takahiko Murata[1,8], Hiroyuki Tanaka[3], Toshiyuki Matsuoka[1], Daiji Sakata[1], Nobuaki Yoshida[4], Koko Katagiri[5], Tatsuo Kinashi[5], Toshiyuki Tanaka[6], Masayuki Miyasaka[6], Hiroichi Nagai[3], Fumitaka Ushikubi[7] & Shuh Narumiya[1]

**Physical interaction of T cells and dendritic cells (DCs) is essential for T cell proliferation and differentiation, but it has been unclear how this interaction is regulated physiologically. Here we show that DCs produce thromboxane $A_2$ ($TXA_2$), whereas naive T cells express the thromboxane receptor (TP). *In vitro*, a TP agonist enhances random cell movement (chemokinesis) of naive but not memory T cells, impairs DC–T cell adhesion, and inhibits DC-dependent proliferation of T cells. *In vivo*, immune responses to foreign antigens are enhanced in TP-deficient mice, which also develop marked lymphadenopathy with age. Similar immune responses were seen in wild-type mice treated with a TP antagonist during the sensitization period. Thus, $TXA_2$-TP signaling modulates acquired immunity by negatively regulating DC–T cell interactions.**

Exposure of dendritic cells (DCs) to foreign antigens initiates immune response. DCs take up and process antigens and migrate toward regional lymph nodes. During migration, DCs are activated and mature[1,2]. Antigen-loaded mature DCs encounter naive T cells in the lymph nodes and make a physical contact referred to as the immunological synapse, through which antigen presentation and associated signaling occur[3–5]. The strength, duration and efficiency of this cell-cell adhesion apparently determine the extent of T cell activation and differentiation, yet little is known about its regulation[4–6].

$TXA_2$ is an unstable metabolite of arachidonic acid produced by catalysis of cyclooxygenase (COX) and thromboxane (TX) synthase in various cell types and exerts its actions through a G-protein-coupled receptor, termed TP[7]. It is produced abundantly by platelets upon exposure to injured blood vessels[8]. Because of its potent platelet-aggregating and vessel-contracting activities, $TXA_2$'s function has been studied mainly in the cardiovascular system[8]. However, we previously found that TP mRNA is highly expressed in the spleen and thymus[9] and that the binding activity for TP in thymocytes is comparable to that in platelets[10]. In addition, TX synthase is enriched in monocytes or macrophages[11–13], which release $TXA_2$ upon activation[14]. Immunohistochemical analysis with antibody to TX synthase also revealed that interdigitating cells with characteristic DC morphology in tissues contain high TX synthase–like immunoreactivity[15]. Together these results implied that $TXA_2$ has a function in the immune system.

We used mice deficient in TP and TP-selective drugs to examine the role of $TXA_2$ signaling in the immune system. Our results show that $TXA_2$-TP signaling negatively regulates DC–T cell interaction and modulates acquired immunity.

## RESULTS

### Generation of TP-deficient mice

We disrupted the gene encoding mouse TP (*Tbxa2r*) by replacing exon 2 and part of exon 3 with the neomycin resistance gene, which resulted in deletion of the region encoding from the N terminus to the sixth transmembrane domain of TP (see **Supplementary Fig. 1** online). The mutated allele was identified by Southern blot analysis and functional disruption of the gene was confirmed by the loss of platelet aggregation in response to a TP agonist, I-BOP[16], as well as by the absence of the ligand-binding activity in thymocytes (**Supplementary Fig. 1**). TP-deficient mice were born morphologically normal at the predicted mendelian frequency. They were fertile, and they showed no abnormalities in blood pressure or heart rate under basal conditions (data not shown), as reported previously[17]. We backcrossed the mutant mice to C57BL/6 or BALB/c mice ten times each. $N_{10}$ (C57BL/6) TP-deficient mice were used in later experiments, with wild-type C57BL/6 inbred mice as controls; $N_{10}$ (BALB/c) TP-deficient mice were used only in mixed lymphocyte reaction (MLR) studies.

### Lymphadenopathy of TP-deficient mice

TP-deficient mice, whether young (8 weeks) or aged (30 weeks), had spleens and thymuses comparable in weight to those of wild-type mice. No difference was found between young TP-deficient and wild-type mice in the numbers of $CD4^+$, $CD8^+$ or $CD4^+CD8^+$ cells in the thymus, $CD4^+$, $CD8^+$, $B220^+$, $CD11b^+$ or $CD11c^+$ cells in the spleen, and immunoglobulin (Ig) $D^+$, $IgM^+$ or $CD43^+$ fractions of $B220^+$ cells in the bone marrow (data not shown). However, marked cervical lymphadenopathy was apparent in aged TP-deficient mice (**Fig. 1a**).

[1]Department of Pharmacology and [2]Department of Dermatology, Kyoto University Faculty of Medicine, Kyoto 606-8501, Japan. [3]Department of Pharmacology, Gifu Pharmaceutical University, Gifu 502-8585, Japan. [4]Institute of Medical Science, University of Tokyo, Tokyo 108-8639, Japan. [5]Department of Molecular Immunology and Allergy, Kyoto University Faculty of Medicine, Kyoto 606-8501, Japan. [6]Molecular and Cellular Recognition, Osaka University Graduate School of Medicine, Suita 565-0871, Japan. [7]Department of Pharmacology, Asahikawa Medical College, Asahikawa 078-8510, Japan. [8]These authors contributed equally to this work. Correspondence should be addressed to S.N. (snaru@mfour.med.kyoto-u.ac.jp).

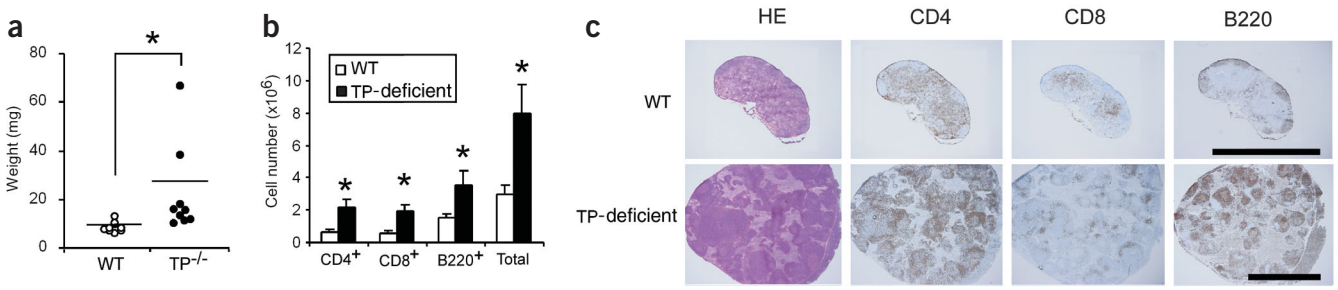


**Figure 1** Lymphadenopathy in aged TP-deficient mice. (**a**) The cervical lymph nodes were isolated from 30-week-old female wild-type (WT) and TP-deficient mice and weighed ($n = 9$, each). (**b**) Numbers of CD4$^+$, CD8$^+$ and B220$^+$ cells of the lymph nodes were measured by flow cytometry. *$P < 0.05$ versus WT mice. (**c**) Serial sections of the cervical lymph nodes were prepared and stained. HE, CD4, CD8 and B220 denote staining with hematoxylin and eosin, anti-CD4, anti-CD8 and anti-B220, respectively. Scale bars, 3 mm.

Accordingly, the numbers of CD4$^+$, CD8$^+$ and B220$^+$ cells in these lymph nodes were significantly higher than in age-matched wild-type mice (**Fig. 1b**). Immunohistochemistry with anti-CD4, anti-CD8 and anti-B220 showed disruption of the zonal structure of the lymph node in TP-deficient mice, and follicles containing T and B cells were found throughout the nodes (**Fig. 1c**). Despite the lymphadenopathy seen in TP-deficient mice, the serum concentrations of IgA, G1, G2a, G2b and G3 remained in the normal range in aged TP-deficient mice (data not shown). More prominent age-related lymphoproliferation, including splenomegaly and enhanced Ig production, was found in the $F_2$ progeny of C57BL/6 × 129Ola TP-deficient mice (see **Supplementary Fig. 2** online).

### Characterization of TP expression and $TXA_2$ production

These findings in aged TP-deficient mice indicated that $TXA_2$ signaling occurs in peripheral lymphoid tissues. Because TP mRNA is highly expressed in the spleen[9] and activated macrophages produce abundant $TXA_2$ (ref. 14), we suspected that $TXA_2$ is formed by antigen-presenting cells (APCs) and acts on lymphocytes. To confirm this, we isolated peripheral lymphocytes, CD11c$^+$ DCs and CD11b$^+$ macrophages and compared TP expression and formation of $TXA_2$—measured as the quantity of a stable metabolite, $TXB_2$—in these cells by RT-PCR. We detected TP mRNA in both CD4$^+$ and CD8$^+$ T cells, but little, if any, in B220$^+$, CD11b$^+$ or CD11c$^+$ cells (**Fig. 2a**). We then cultured CD4$^+$, B220$^+$, CD11b$^+$ and CD11c$^+$ cells separately and measured their production of $TXA_2$. CD4$^+$ T cells and B220$^+$ B cells each produced only negligible amounts of $TXA_2$ by 24 h of culture, and this production was not enhanced by activation of the cells with anti-CD3 or bacterial lipopolysaccharide (LPS), respectively. On the other hand, both CD11b$^+$ macrophages and CD11c$^+$ DCs produced abundant $TXA_2$, and activation of these cells with LPS further augmented their $TXA_2$ production (**Fig. 2b**). Production by CD11c$^+$ DCs was higher than that by CD11b$^+$ macrophages on a per-cell basis. Because resting macrophages produce little $TXA_2$ without stimulation[14], we presume that the CD11b$^+$ and CD11c$^+$ cells used were already activated before incubation, possibly by linkage of their CD11 integrins with the respective antibodies during preparation. It is known that $TXA_2$ production by macrophages is associated with cell adherence to the matrix[18]. We found no difference in the generation of $TXA_2$ by CD11c$^+$ DCs from wild-type versus TP-deficient mice (data not shown). These results indicate that $TXA_2$ signaling can occur in the periphery, with $TXA_2$ being produced by activated DCs and acting on TP expressed by T cells.

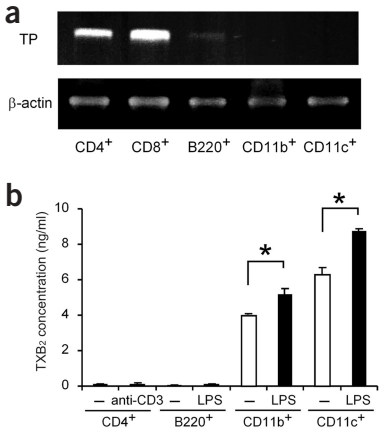


**Figure 2** TP expression and $TXA_2$ production. (**a**) RT-PCR analysis for TP mRNA expression. CD4$^+$, CD8$^+$, B220$^+$, CD11b$^+$ and CD11c$^+$ cells were purified from spleens of wild-type (WT) mice and subjected to RT-PCR. β-actin was used as an internal control. (**b**) Production of $TXA_2$, as measured as a stable metabolite, $TXB_2$. CD4$^+$, B220$^+$, CD11b$^+$ and CD11c$^+$ cells purified from the spleen of WT mice were incubated with or without stimulation for 24 h (see Methods). The amount of $TXB_2$ in the culture medium was determined in triplicate by ELISA. *$P < 0.05$ versus vehicle-treated cells. Data shown are the representative of at least three experiments.

### $TXA_2$ inhibits DC-induced T cell activation

Our findings demonstrated that the loss of the $TXA_2$ signaling results in expansion of the peripheral lymphoid tissues and indicated that this signaling axis might either down-regulate proliferation or enhance apoptosis of T cells in the periphery. Because both CD4$^+$ and CD8$^+$ cells express TP (**Fig. 2a**) and increase in number in the absence of TP signaling (**Fig. 1b**), we further analyzed CD4$^+$ cells as representatives of T cells. We first examined the effect of a TP agonist, I-BOP, on the cells' proliferation *in vitro*. We used 1 μM I-BOP, a concentration known to produce $TXA_2$-induced biological responses such as platelet aggregation (**Supplementary Fig. 1**). We also added a COX inhibitor, indomethacin, to inhibit actions of endogenous prostanoids. Treatment with I-BOP did not inhibit the DC-independent CD4$^+$ T cell proliferation induced by phorbol myristate acetate (PMA) and ionomycin (**Fig. 3a**). This stimulation mimics intracellular signaling evoked by T cell activation and bypasses all the biological processes leading to the activation[19], whereas, physiological T cell activation and proliferation depend on the T cells' interaction with DCs.

To examine whether $TXA_2$-TP signaling functions in DC-dependent T cell activation, we constructed an *in vitro* system for DC-supported antigen-specific T cell proliferation. CD4$^+$ cells were purified

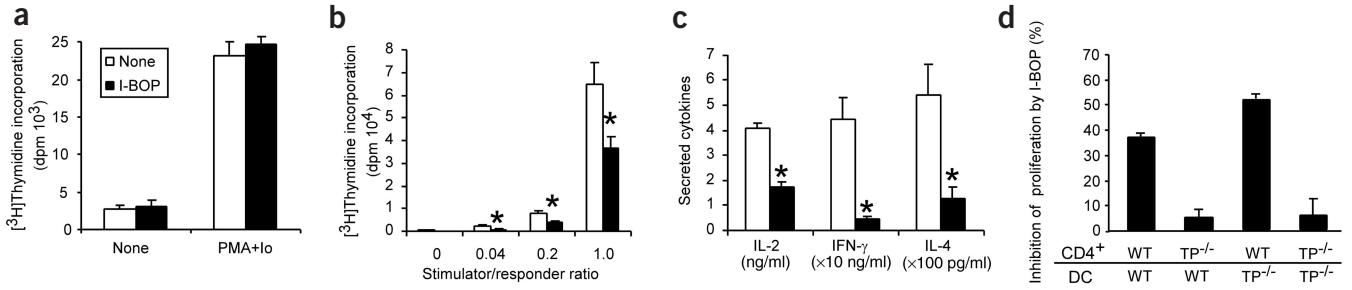


**Figure 3** Inhibition of DC-dependent T cell proliferation by I-BOP. (**a**) Effect of I-BOP on CD4$^+$ cell proliferation by PMA and ionomycin (Io). CD4$^+$ cells were stimulated with or without PMA and ionomycin (PMA+Io) in the presence or absence of I-BOP, and incorporation of [$^3$H]thymidine was measured. (**b**,**c**) Effect of I-BOP on antigen-specific CD4$^+$ cell proliferation and cytokine production. CD4$^+$ cells from DO11.10 mice (responder) were incubated with different numbers of CD11c$^+$ cells from BALB/c mice (stimulator) and OVA in the presence of vehicle or I-BOP. [$^3$H]Thymidine incorporation (**b**) and the amounts of IL-2, IFN-γ and IL-4 in the culture medium at the stimulator/responder ratio of 1 (**c**) were measured. *$P < 0.05$ versus vehicle-treated cells. (**d**) Resistance of CD4$^+$ cells from TP-deficient mice (TP$^{-/-}$) to inhibition by I-BOP. An MLR was performed with the indicated pairs of CD4$^+$ cells and CD11c$^+$ DCs from TP$^{-/-}$ and wild-type (WT) mice (CD4$^+$ T cells were derived from C57BL/6 mice, DCs derived from BALB/c mice), and the growth inhibitory effect of I-BOP was compared. The percentage inhibition by I-BOP is shown. Data shown are the representatives of at least three experiments.

from DO11.10 transgenic mice overexpressing the T cell receptor (TCR) and recognizing the amino acid 323–339 peptide of ovalbumin (OVA) and mixed with DCs from syngeneic BALB/c mice in the presence of OVA. OVA-dependent proliferation was then determined with or without I-BOP. We found that I-BOP suppressed this proliferation at all CD11c$^+$/CD4$^+$ cell ratios examined (**Fig. 3b**). In addition, the I-BOP treatment significantly suppressed interleukin (IL)-2, interferon (IFN)-γ and IL-4 secretion by CD4$^+$ cells at a stimulator/responder ratio of 1 (**Fig. 3c**), indicating that TP stimulation might suppress both proliferation and differentiation of T cells. Similar suppression of T cell proliferation by I-BOP was observed in an MLR study, in which CD4$^+$ cells from C57BL/6 mice were cocultured with CD11c$^+$ cells from BALB/c mice at various cell ratios (data not shown). T cell proliferation in an MLR depends on direct recognition by TCR of allogeneic major histocompatibility antigen complex (MHC) on DCs and is thought to mimic antigen-dependent interaction between syngeneic DCs and naive T cells[20,21]. In the MLR, the I-BOP treatment also significantly ($P < 0.05$) suppressed IL-2 and IFN-γ production by CD4$^+$ cells (data not shown). Taking advantage of the MLR, we next determined on which type of cells I-BOP acted to suppress proliferation. By incubating T cells from wild-type or TP-deficient C57BL/6 mice with DCs from either wild-type or TP-deficient BALB/c mice, we found that, irrespective of the origin of cocultured DCs, I-BOP inhibited proliferation of wild-type but not TP-deficient T cells (**Fig. 3d**). These results indicate that I-BOP acts on TP in T cells and inhibits proliferation. We also found that I-BOP neither increased the number of apoptotic cells in the MLR nor enhanced activation-induced cell death of concanavalin A–primed T cells restimulated with anti-CD3 (ref. 22) (data not shown), thus excluding a possibility that I-BOP enhanced apoptosis to produce the above effects. These results indicate that $TXA_2$-TP signaling might inhibit DC-dependent CD4$^+$ cell proliferation and differentiation.

### $TXA_2$ suppresses DC–T cell interaction

We noticed that the clusters of CD4$^+$ cells formed around DCs were smaller and flatter in the presence of I-BOP in both the OVA-specific proliferation and MLR assays (**Fig. 4a**). This attenuation by I-BOP was not observed in the MLR of CD4$^+$ cells from TP-deficient mice, whereas DCs from TP-deficient mice triggered T cell clustering as much as wild-type DCs and I-BOP attenuated wild-type T cell clustering induced by DCs from TP-deficient mice (data not shown). As reported previously[23], an attenuation of DC–T cell clusters similar to that induced by I-BOP was observed in the presence of antibodies to lymphocyte function–associated antigen (LFA)-1 (**Fig. 4a**). Because LFA-1 binds to its counter-receptor, intercellular adhesion molecule (ICAM)-1, and stabilizes DC–T cell adhesion[23], these results indicated that TP stimulation by I-BOP might affect adhesion between DCs and T cells. To test this hypothesis, we further evaluated the DC–T cell adhesion by quantifying the formation of conjugates between CD4$^+$ cells from C57BL/6 mice and CD11c$^+$ DCs from BALB/c mice. Coincubation of the allogeneic CD4$^+$ and CD11c$^+$ cells resulted in conjugate formation, which was significantly decreased by the addition of I-BOP (**Fig. 4b**). No such decrease occurred when CD4$^+$ cells from TP-deficient mice were used. These results seemed to indicate that TP stimulation in CD4$^+$ cells interfered with formation of the conjugates. To further analyze the mechanism whereby TP signaling impaired T cell–DC adhesion, we plated CD4$^+$ cells on ICAM-1-coated dishes and stimulated them with PMA to induce LFA-1 activation[24]. Actin filaments were visualized by phalloidin staining. The PMA-stimulated CD4$^+$ cells adhered to ICAM-1-coated plates and took on an extended, polarized morphology, as evidenced by the accumulation of filamentous actin (F-actin) on one side of the cells (**Fig. 4c**,**d**). With anti–LFA-1, we verified that these changes were dependent on LFA-1, as previously reported[24]. The addition of I-BOP to these extended cells caused marked cell detachment; the cells became round, with thick actin bundles encircling the cell body, indicating that I-BOP induced cell contraction and disrupted T cell adhesion to ICAM.

### $TXA_2$ promotes chemokinesis of naive T cells

Our findings seemed to indicate that T cells can dissociate from DCs upon TP stimulation. It has been suggested that the delivery of 'go' signals for T cell migration is essential to the termination of DC–T cell interaction *in vivo*[25,26]. We therefore used a transwell assay to examine whether the effect of TP stimulation that we had observed is related to CD4$^+$ cell motility. Addition of I-BOP either to the upper chamber with CD4$^+$ cells, to the lower chamber or to both chambers stimulated cell migration to the lower chamber significantly (**Fig. 5a**). Flow cytometric analysis showed that the cells that responded to I-BOP were CD4$^+$CD44$^-$ naive cells. However, the number of migrating CD4$^+$CD44$^+$ memory cells was the same with or without I-BOP. This enhancement of naive CD4$^+$ cell migration by I-BOP was not observed in TP-deficient mice and was counteracted completely by treatment with the TP antagonist SQ-29548 (ref. 7) (**Fig. 5b**). This effect of I-BOP seemed to result from enhancement of random cell movement (chemokinesis), because the migration did not

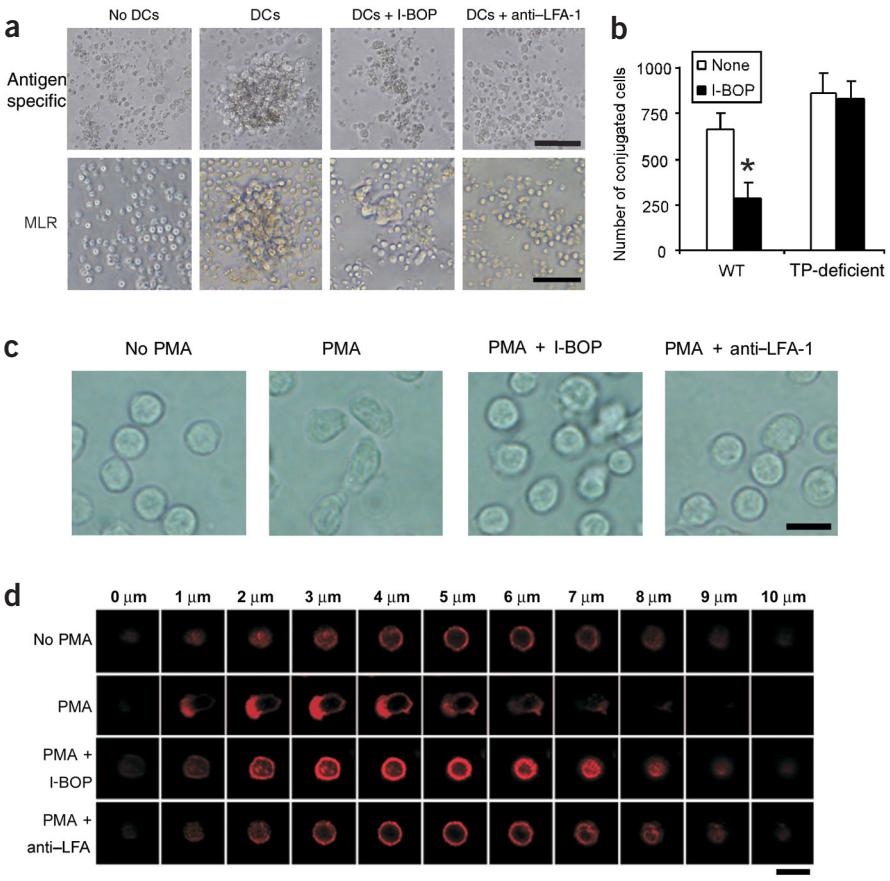


**Figure 4** Inhibition of T cell adhesion by I-BOP. (**a**) Effect of I-BOP on cluster formation and appearance. $CD4^+$ cells from DO11.10 mice or wild-type (WT) C57BL/6 mice were incubated with or without $CD11c^+$ DCs from BALB/c mice in the presence or absence of I-BOP or anti–LFA-1 for 72 h, and cell clusters were photographed under phase-contrast microscopy. OVA was added as an antigen in the upper experiments. Scale bars, 100 μm. (**b**) Effect of I-BOP on formation of DC–T cell conjugates. BALB/c DCs were incubated with $CD4^+$ cells from either WT or TP-deficient C57BL/6 mice in the presence or absence of I-BOP for 60 min at 37 °C, and conjugated cells were quantified by flow cytometry. $*P < 0.05$ versus vehicle-treated group. Data shown are the representatives of at least three experiments. (**c**,**d**) Effect of I-BOP on T cell adhesion to ICAM-1-coated plates. $CD4^+$ cells were stimulated with PMA for 60 min on an ICAM-1-coated plate and then treated with either I-BOP or anti–LFA-1. The cells were examined by phase contrast microscopy (**c**) or fixed and stained with Texas Red–labeled phalloidin (**d**). Optical sections obtained by confocal microscopy are shown from bottom to top in **d**. Scale bars, 10 μm.



depend on the concentration gradient of I-BOP. To determine why I-BOP differentially affected naive and memory cells, we examined the expression of TP on $CD4^+CD44^-$ naive cells and $CD4^+CD44^+$ memory cells. RT-PCR analysis showed that TP was expressed strongly in naive cells but only weakly in memory cells (**Fig. 5c**). In addition, activation of memory cells did not induce expression of TP mRNA or production of $TXA_2$. These results indicated that $TXA_2$-TP signaling might stimulate chemokinesis of $CD4^+CD44^-$ naive cells to negatively regulate the interaction between DCs and T cells. If this conclusion is correct, I-BOP should have no effect on conjugate formation by memory T cells. We tested this hypothesis using 3A9 hen egg lysozyme (HEL)-specific, $I\text{-}A^k$-restricted T cell hybridoma[27]. 3A9 T cell hybridoma cells were incubated with CH-27 B lymphoblastoid cells in the presence of HEL with or without I-BOP at 37 °C for 60 min, and conjugate formation was examined by flow cytometry. This line of T cells, which shows little TP expression (data not shown), represents activated T cells and forms conjugates with CH-27 B-lymphoblastoid cells expressing $I\text{-}A^k$ MHC[28]. Addition of I-BOP did not inhibit HEL-specific conjugate formation between the two cell lines (data not shown).

### Enhanced contact hypersensitivity in TP-deficient mice

We next assessed the *in vivo* significance of these *in vitro* findings. We used a contact hypersensitivity (CHS) approach to determine whether blockade of TP signaling evokes altered immune responses to an exogenous antigen. The CHS model consists of two phases. The sensitization phase, lasting about 3 d, begins with antigen exposure to the skin, which results in antigen uptake by DCs, migration of the DCs to draining lymph nodes, and antigen presentation. This activates naive T cells, resulting in the generation of effector T cells. The elicitation phase is initiated by a second application of antigen to the skin (the challenge) about 5 d after the primary exposure. The applied antigen recruits and activates effector T cells locally to induce a $T_H1$-dependent local inflammation (the hypersensitivity response) that peaks at 24–48 h after the second exposure. We sensitized 8-week-old mice by administration of dinitrofluorobenzene (DNFB) on the abdomen and challenged them 5 d later by DNFB application to the ear[29]. TP-deficient mice showed significantly greater ear swelling than wild-type mice (**Fig. 6a**), and the difference was reproduced at different concentrations of antigen (data not shown). To confirm that this difference was due to the TP deficiency and not to other genetic factors, we administered the TP antagonist S-145 (ref. 30) to wild-type mice, either throughout the experiment or selectively during either the sensitization or elicitation period. Wild-type mice treated with S-145 showed significantly greater ear swelling, of the same magnitude as that found in TP-deficient mice (**Fig. 6a**). However, this enhanced responsiveness occurred only when S-145 was administered during sensitization, not elicitation. These results strongly indicate that endogenously produced $TXA_2$ acts during sensitization to modulate the immune response of the animals but has little effect during elicitation.

To confirm that this modulation of the immune response occurred through sensitization of T cells, we analyzed T cell populations in the draining lymph nodes of wild-type and TP-deficient mice 5 d after DNFB application to the abdomen. The DNFB treatment increased the sizes of regional lymph nodes and the numbers of $CD4^+$, $CD8^+$ and $B220^+$ cells in the lymph nodes, for both wild-type and TP-deficient mice. However, the proliferative response in the lymph nodes was greater in TP-deficient mice than in wild-type mice, and each cell

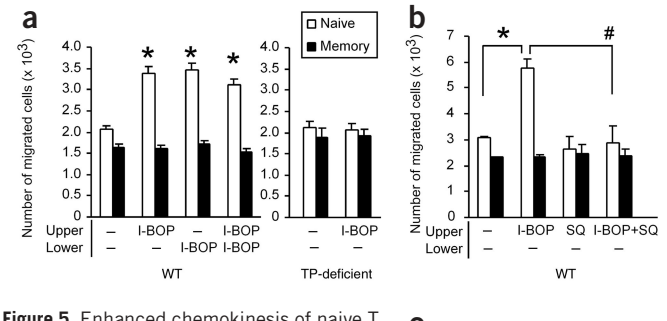


**Figure 5** Enhanced chemokinesis of naive T cells by I-BOP. (**a**) Transwell assay. $CD4^+$ cells from wild-type (WT) or TP-deficient C57BL/6 mice pretreated with or without I-BOP for 1 h were transferred together to the upper chamber of the transwell and incubated for 3 h. In some experiments, I-BOP was added to the lower chamber. The number of $CD4^+CD44^-$ naive or $CD4^+CD44^+$ memory cells that migrated to the lower chamber were analyzed by flow cytometry. $^*P < 0.05$ versus vehicle-treated group. (**b**) Inhibition of I-BOP-induced migration of $CD4^+CD44^-$ naive cells by a TP antagonist. The above experiments were performed in the presence or absence of a TP antagonist, SQ-29548. $\#P < 0.05$ versus the group treated with I-BOP alone. (**c**) TP expression in naive and not memory T cells. $CD4^+CD44^-$ naive or $CD4^+CD44^+$ memory cells were sorted and TP expression was examined by RT-PCR. The $CD4^+CD44^+$ memory cells were also activated with anti-CD3 and added to the analysis. Expression of β-actin was used as an internal control. Data shown are the representatives of at least three experiments.

subset was proportionately more populous in the TP-deficient mice (**Fig. 6b**). Among both the $CD4^+$ and the $CD8^+$ cell populations in the lymph nodes, the numbers of $CD44^+$ memory cells were about twice as high in TP-deficient mice as in wild-type mice (**Fig. 6c**). We further examined the antigen-specific memory T cell population in the lymph nodes by measuring proliferative response to 2,4-dinitrobenzenesulfonic acid (DNBS), a water-soluble compound with the same antigenicity as DNFB. Significantly higher proliferative responses were found in cells from the lymph nodes of TP-deficient mice than in those from wild-type mice (**Fig. 6d**). Consistently, a significantly higher number of effector cells bearing the $T_H1$ cytokine IFN-γ was detected in the cervical lymph nodes of TP-deficient mice than in those of wild-type mice 24 h after challenge (**Fig. 6e**). IFN-γ-bearing cells consisted of $CD4^+$, $CD8^+$ and $B220^+$ cells, and their major constituent fraction was $CD8^+$ cells, which are the important effector T cells in CHS[1]. These results indicate that the $TXA_2$ endogenously produced during sensitization acts *in situ* in the lymph nodes to limit T cell priming.

We next subjected wild-type and TP-deficient mice to repetitive DNFB ear challenge. In addition to the delayed-type reaction as seen in CHS, repetitive DNFB challenge induces immediate-type hypersensitivity, which is mediated by $T_H2$ lymphocytes and consists of early- and late-phase reactions[31]. Before this experiment, we confirmed that there is no substantial difference in the numbers of mast cells and eosinophils in the skin and blood of wild-type and TP-deficient mice under basal conditions (data not shown). We also determined that a passive cutaneous anaphylaxis reaction elicited by anti-dinitrophenol IgE was no greater in TP-deficient mice than in wild-type mice (data not shown), indicating that the responsiveness of mast cells is similar in the two strains. For the repetitive ear challenge, mice were painted with 20 μl of 0.2% DNFB on the right ear once per week for 4 weeks. The extent of each reaction was evaluated by measuring ear thickness 1, 4 and 24 h after each application. The immediate-type reaction increased in intensity on repeated challenge, and after the fourth challenge, it was significantly greater in TP-deficient mice than in wild-type mice (**Fig. 7a**). Histological examination of the DNFB-treated ears of TP-deficient mice revealed a marked thickening of the epidermis and augmented infiltration of inflammatory cells, including lymphocytes, neutrophils, mast cells and eosinophils (**Fig. 7b**). Consistently, infiltration of cells showing IgE staining occurred, and this was more pronounced in the ears of TP-deficient mice than in those of wild-type mice (**Fig. 7c**). However, serum IgE concentrations were below the limit of detection in both wild-type and TP-deficient mice, which probably reflected the weak $T_H2$ response in the $T_H1$-dominant C57BL/6 mice. Enhanced ear swelling was greater and serum IgE concentrations were significantly higher in TP-deficient individuals among the $F_2$ progeny of the C57BL/6 × 129Ola cross (see **Supplementary Fig. 3** on line). These results indicate that TP deficiency causes substantial enhancement of both $T_H1$ and $T_H2$ immune responses to foreign antigens.

## DISCUSSION

By studying mice deficient in TP as well as TP-selective drugs, we have shown here that $TXA_2$-TP signaling negatively regulates

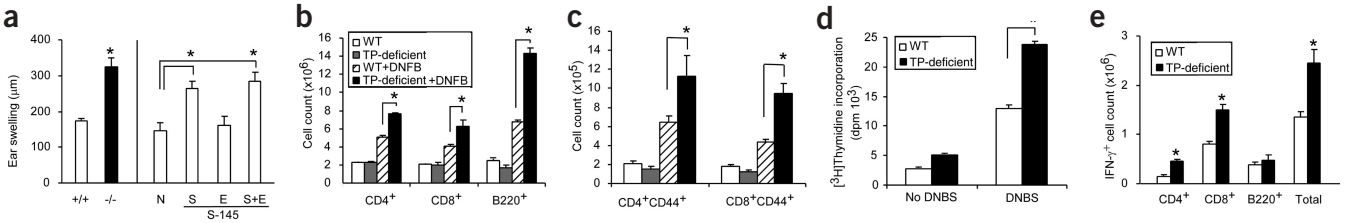


**Figure 6** Enhanced CHS responses in TP-deficient mice. (**a**) Ear thickness. Wild-type (+/+) and TP-deficient (–/–) mice were immunized with DNFB and challenged 5 d later, and their ear thickness was measured 24 h after the challenge ($n = 6$, left). Wild-type mice were treated either with vehicle (N) or S-145 during sensitization (S), during elicitation (E) or all through the experimental period (S+E), and ear thickness was measured ($n = 6$, right). $^*P < 0.05$ versus wild-type (WT) or vehicle-treated mice. (**b**,**c**) Quantitative analysis of lymphocyte subsets of the regional lymph nodes. The regional lymph nodes were isolated from WT and TP-deficient C57BL/6 mice 5 d after treatment with (+DNFB) or without DNFB, and the numbers of $CD4^+$, $CD8^+$ and $B220^+$ cells (**b**) or $CD4^+CD44^+$ and $CD8^+CD44^+$cells (**c**) were measured. $^*P < 0.05$ versus WT mice with DNFB treatment. (**d**) DNBS-induced lymphocyte proliferation. Lymphocytes were purified from cervical lymph nodes of wild-type and TP-deficient mice 24 h after the DNFB challenge and incubated for 3 d with or without DNBS, and proliferation was measured by [$^3$H]thymidine incorporation. $^*P < 0.05$ versus wild-type mice with DNBS treatment. (**e**) Analysis of IFN-γ-producing cells. $CD4^+$, $CD8^+$ or $B220^+$ cells were obtained from cervical lymph nodes of wild-type and TP-deficient mice 24 h after the challenge and stained for intracellular IFN-γ. The numbers of IFN-γ -positive cells are shown. $^*P < 0.05$ versus wild-type mice. Data shown are the representatives of at least three experiments.

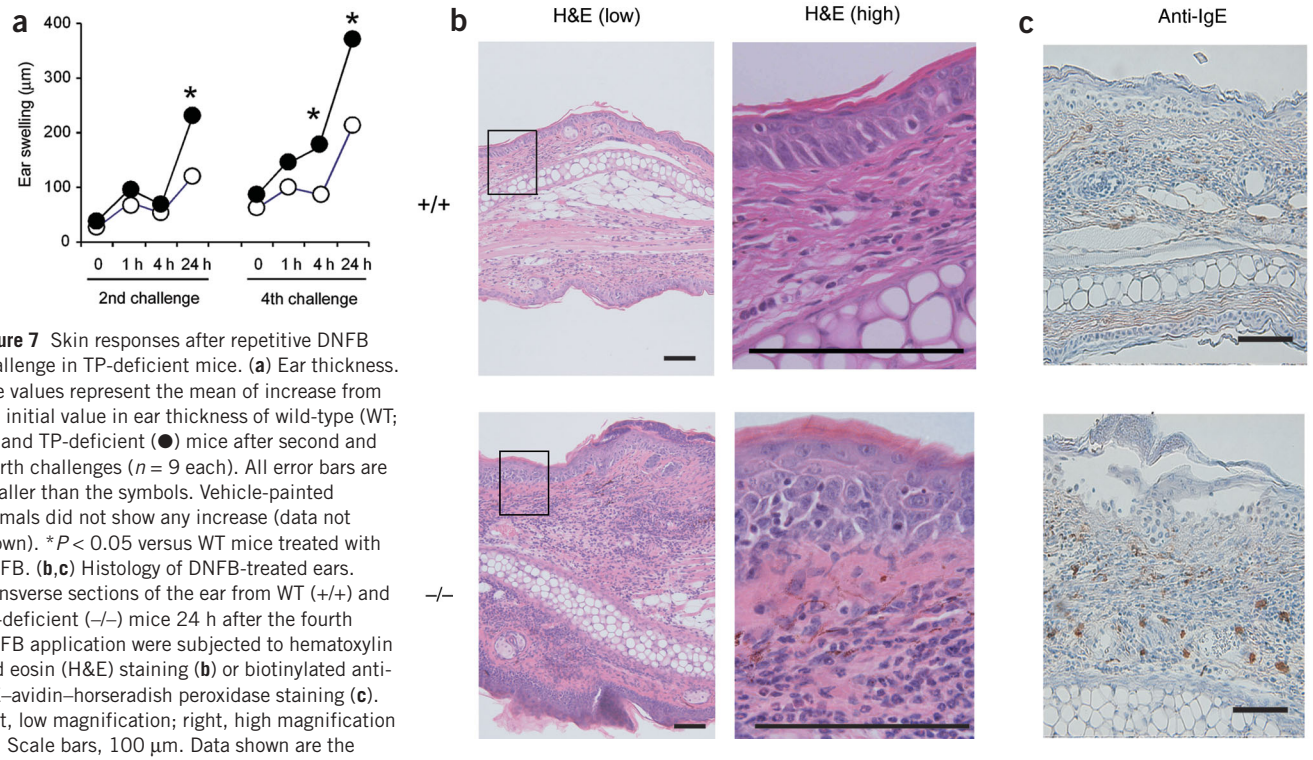


**Figure 7** Skin responses after repetitive DNFB challenge in TP-deficient mice. (**a**) Ear thickness. The values represent the mean of increase from the initial value in ear thickness of wild-type (WT; ○) and TP-deficient (●) mice after second and fourth challenges ($n = 9$ each). All error bars are smaller than the symbols. Vehicle-painted animals did not show any increase (data not shown). *$P < 0.05$ versus WT mice treated with DNFB. (**b**,**c**) Histology of DNFB-treated ears. Transverse sections of the ear from WT (+/+) and TP-deficient (–/–) mice 24 h after the fourth DNFB application were subjected to hematoxylin and eosin (H&E) staining (**b**) or biotinylated anti-IgE–avidin–horseradish peroxidase staining (**c**). Left, low magnification; right, high magnification (**b**). Scale bars, 100 μm. Data shown are the representatives of at least three experiments.



immune responses to exogenous antigens through modulation of interactions between DCs and T cells. $TXA_2$ binds to TP and induces a variety of cellular responses. $TXA_2$ is known to activate platelets, cause smooth muscle contraction[32] and induce chemokinesis of endothelial cells[33]. Here, we have found that TP stimulation induces strong contraction, abolishes polarized F-actin accumulation, disrupts LFA-1-mediated adhesion to ICAM-1-coated plates, enhances chemokinesis, and suppresses proliferation of $CD4^+$ cells. Because the interaction of DCs and T cells is dependent on the interaction of LFA-1 and ICAM-1 and is maintained by polarized accumulation of F-actin[24,34–36], we suggest that TP stimulation attenuates the DC–T cell interaction by depolarizing the actin cytoskeleton and promoting random migration of T cells.

How, then, can $TXA_2$ be produced and trigger TP signaling during immune responses? We have shown here that $CD11c^+$ DCs and $CD11b^+$ macrophages, isolated by antibody ligation of their integrins, potently produce $TXA_2$. Integrin activation is associated with activation and migration of DCs and macrophages[37,38], and $TXA_2$ is produced by macrophages in response to a variety of stimuli including cytokines[39,40], phagocytosis[14,41] and cell-cell interaction[42]. For example, proinflammatory cytokines such as tumor necrosis factor (TNF)-α and IL-1β activate macrophages to produce $TXA_2$ for hours. These proinflammatory cytokines are produced in association with antigen exposure and activate DCs. Indeed, LPS, a proinflammatory stimulus, augmented $TXA_2$ production by these cells. It is therefore likely that DCs are activated by various mechanisms to produce $TXA_2$ constitutively after antigen exposure.

It is noteworthy that enhanced chemokinesis by I-BOP is seen only in naive T cells but not in memory cells, and that the TP expression seen in naive cells was greatly suppressed in memory cells. These results indicate that $TXA_2$ produced by DCs may limit the number of naive T cells interacting with DCs and/or determine the strength and duration of this interaction, thereby adjusting the extent of acquired immunity. We have consistently found augmentation of memory T cell formation in the absence of TP signaling *in vivo*. Although our present study focused on the role of $TXA_2$ in the DC–T cell interaction, $TXA_2$-TP signaling may well modulate the interaction of naive T cells with other types of APCs, such as macrophages, given that a sufficient amount of $TXA_2$ is generated during such interaction.

Actions of TP in the immune system other than those presented here have been proposed previously: specifically, inhibition of CD26 dipeptidase by the N-terminal peptide of TP protein[43] and up-regulation of adhesion molecules in vascular endothelial cells by platelet-derived $TXA_2$ (ref. 44). However, the former is physiologically unlikely because of the heavy glycosylation of the native receptor and the requirement of very high peptide concentration, and the latter effect is only seen in the elicitation phase in mast cell–deficient mice. In contrast, TP-mediated attenuation of the DC–T cell interaction is physiologically relevant, being consistent with the phenotype of TP-deficient mice, the *in vivo* action of the TP antagonist and the findings from our *in vitro* experiments. Our study has thus opened a new avenue of research into APC–T cell interactions. A current focus in this field is elucidation of the molecular architecture of the interaction and subsequent intracellular signaling, and little is known about how the interaction is regulated physiologically. A certain group of chemokines has been implicated in this process but only from an *in vitro* study[25]. Our study has demonstrated that a completely different category of molecule can regulate the DC–T cell interaction physiologically. This finding should be taken into account in dissecting the pathogenesis of various immunological disorders. In summary, we have identified $TXA_2$-TP signaling as a negative regulator of acquired immunity. Because $TXA_2$ is formed and released by activated DCs, this system could be envisioned as a built-in 'off' switch associated with DC activation.

## METHODS

**Generation of TP-deficient mice.** Genomic clones carrying the gene encoding TP were isolated from a mouse genomic DNA library with TP cDNA as a probe. Disruption of the gene was done as described in Results. Two independent embryonic stem cell lines were established, and chimeric male mice generated from these lines were backcrossed with C57BL/6 females. The resulting heterozygous littermates were mated to produce homozygous TP-deficient mice. TP-deficient mice were backcrossed ten times with C57BL/6 or BALB/c mice. Mice were maintained on a 12 h light–12 h dark cycle under specific pathogen–free conditions. All experimental procedures were approved by the Committee on Animal Research of Kyoto University Faculty of Medicine.

**Histology, immunofluorescence and flow cytometry.** Tissue sections were prepared and stained for CD4, CD8 and B220 as described previously[45]. Rat anti–mouse IgE (LO-ME, Immunotech) was also used. For visualization of F-actin, cells were fixed in 4% paraformaldehyde, permeabilized with 0.7% saponin for 30 min at room temperature, and stained with a 1/200 dilution of Texas Red–labeled phalloidin (Molecular Probes). Images of cells were taken on the Zeiss Confocal Laser Scanning Unit (LSM 510-V2.5).

Cells were stained with monoclonal antibodies conjugated to fluorescein isothiocyanate (FITC) or phycoerythrin and then subjected to flow cytometry on an EPICS XL (Beckman Coulter).

**Cell preparation and culture.** Spleen cells were dispersed and purified to $CD4^+$, $CD8^+$, $B220^+$, $CD11b^+$ or $CD11c^+$ populations by auto MACS (Miltenyi Biotec) or sorted into $CD4^+CD44^-$ or $CD4^+CD44^+$ cells by FACS Vantage (Becton Dickinson). The purity of each population was confirmed to be >95% by flow cytometry. Cells were cultured in flat-bottomed 96-well plates at 37 °C in an atmosphere of 5% $CO_2$ in phenol red–free RMPI 1640 (Life Technologies) containing 10% fetal bovine serum (FBS), 100 U/ml penicillin, 100 μg/ml streptomycin, 300 mg/ml L-glutamine, 50 μM β-mercaptoethanol and 1 mM sodium pyruvate.

For measurement of $TXB_2$, $2 \times 10^5$ $CD4^+$ cells were incubated for 24 h with plate-bound anti-CD3 (10 μg/ml) in 200 μl of the culture medium without FBS. Similarly, $2 \times 10^5$ B220 cells, $2 \times 10^5$ $CD11b^+$ cells or $4.5 \times 10^4$ $CD11c^+$cells were incubated for 24 h with LPS (1 μg/ml). $TXB_2$ in the culture medium was measured with an enzyme immunoassay (EIA) kit (Cayman Chemical). RT-PCR analysis for TP was performed on total RNA with the sense primer 5′-TGCCTTGTTGGACTGGCGAGCCACTGACCC-3′, the antisense primer 5′-CAGGTAGATGAGCAGCTGGTGCTCTGTGGC-3′ and the SuperScript One-Step RT-PCR System (Invitrogen, Carlsbad, CA). Proliferation was induced with PMA and ionomycin by incubating $2 \times 10^5$ $CD4^+$ cells with 10 ng /ml PMA (Sigma Chemical) and 1 μM ionomycin (Wako) for 72 h in the culture medium supplemented with 10 μM indomethacin. The cells were pulsed with 1 μCi [$^3$H]thymidine for the last 24 h of culture. Incorporated [$^3$H]thymidine was measured with a liquid scintillation counter (Aloka Co. Ltd.). For antigen-specific cell proliferation, $2 \times 10^5$ $CD4^+$ cells from the spleen of DO11.10 mice were cultured with 100 ng/ml of OVA and irradiated syngeneic $CD11c^+$ cells from the spleen of BALB/c mice for 96 h. The amounts of IL-2, IFN-γ, and IL-4 at 72 h of culture at a $CD11c^+/CD4^+$cell ratio of 1 were measured by EIA kits (Endogen) as described[45]. For MLR, $2 \times 10^5$ $CD4^+$ T cells from the spleens of wild-type or TP-deficient C57BL/6 ($N_{10}$) mice were incubated with irradiated $CD11c^+$ DCs from the spleens of wild-type or TP-deficient BALB/c ($N_{10}$) mice in the culture medium supplemented with 10 μM indomethacin in the presence or absence of 1 μM I-BOP ([1S-(1α,2β(5*Z*),3α(1*E*,3*S**),4α)]-7-[3-(3-hydroxy-4-(4′-iodophenoxy)-1-butenyl)-7-oxabicyclo-[2.2.1]heptan-2-yl]-5-heptenoic acid) for 96 h. The cells were pulsed with 1 μCi [$^3$H]thymidine for the last 24 h of culture and cell proliferation was measured. For adhesion assay, plastic dishes were coated with mouse ICAM-1–Ig chimera (Genzyme Techne)[24]. $CD4^+$ cells ($1.5 \times 10^6$/ml) were plated and then incubated with or without PMA for 1 h before treatment with or without 1 μM I-BOP.

**Conjugate formation.** $CD11c^+$ DCs from wild-type or TP-deficient BALB/c ($N_{10}$) mice were labeled with PKH-26 (Sigma). $CD4^+$ T cells from wild-type or TP-deficient C57BL/6 ($N_{10}$) mice were labeled with 5,6-carboxyfluorescein diacetate (Molecular Probes). Labeled T cells and DCs ($1 \times 10^5$ cells each) were mixed in culture medium supplemented with 10 μM indomethacin and centrifuged at 50*g* for 1 min. The pellet was then incubated for 60 min at 37 °C. Nonspecific aggregates were disrupted by vortexing and the samples were analyzed by flow cytometry. The number of conjugated cells was defined as double-positive events in the upper right quadrant among 50,000 events[24].

**Transwell assay.** $CD4^+$ cells ($3 \times 10^5$ cells) were preincubated in 100 μl medium with or without TP-selective drugs for 1 h. They were then transferred with the medium to 5-μm-pore-size polycarbonate 24-well tissue culture inserts (Costar), with 600 μl of medium in the lower well, and incubated for 3 h at 37 °C. In some experiments, TP-selective drugs or the TP antagonist SQ-29548 ([1S-[1α,2α(Z),3α,4α]]-7-[3-[[2-[(phenylamino)carbonyl]hydrazino]methyl]-7-oxabicyclo[2,2,1]hept-2-yl]-5-heptenoic acid) were also added to the lower wells. The numbers of $CD4^+CD44^-$ naive and $CD4^+CD44^+$ memory cells that migrated to the lower chamber were determined by flow cytometry.

**DNFB-induced contact hypersensitivity.** Female mice 8 weeks of age were immunized by application of 25 μl of 0.5% DNFB in 4:1 (vol/vol) acetone/olive oil to their shaved abdomens on day 0. They were challenged on the right ear on day 5 with 20 μl of 0.3% (wt/vol) DNFB[29]. Ear thickness was measured before and 24 h after challenge to assess inflammation. For treatment with S-145 (5-(+)-(*Z*)-7-[(1*R*, 2*S*, 3*S*, 4*S*)-3-phenylsulfonylaminobicyclo[2.2.1]hept-2-yl]-heptenoic acid), the compound was administered orally in drinking water (200 mg/l) during the sensitization period (from 1 d before the DNFB sensitization to 3 d after the DNFB sensitization) or elicitation period (from 1 d before challenge to 1 d after challenge). The dose administered was calculated to be ~50 mg per kg body weight per day. For a repetitive challenge model, mice were subjected to topical application of 20 μl of 0.2% DNFB on the right ear once per week for 4 weeks[31].

For DNBS-dependent proliferation, cells were prepared from axillary and inguinal lymph nodes 5 d after the DNFB sensitization. $4 \times 10^5$ cells were cultured with DNBS sodium (50 μg/ml) for 3 d, and were pulsed with 1 μCi [$^3$H]thymidine for the last 24 h of culture. Intracellular cytokine staining was performed as described[46] with phycoerythrin-conjugated rat anti–mouse IFN-γ (PharMingen) on cells obtained from the cervical lymph nodes ($1 \times 10^6$/ml) 24 h after challenge with DNFB.

**Statistical analysis.** All the experiments were performed at least three times, and representative results are shown. Data are presented as the means ± s.e.m. Data were analyzed with an unpaired 2-tailed *t*-test or 1-way analysis of variance followed by the Tukey multiple comparisons. *P* values <0.05 were considered statistically significant.

*Note: Supplementary information is available on the Nature Immunology website.*

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1. Grabbe, S. & Schwarz, T. Immunoregulatory mechanisms involved in elicitation of allergic contact hypersensitivity. *Immunol. Today* **19**, 37–44 (1998).
2. Lanzavecchia, A. & Sallusto, F. Regulation of T cell immunity by dendritic cells. *Cell* **106**, 263–266 (2001).
3. Paul, W.E. & Seder, R.A. Lymphocyte responses and cytokines. Cell 76, 241–251 (1994).
4. Monks, C.R., Freiberg, B.A., Kupfer, H., Sciaky, N. & Kupfer, A. Three-dimensional segregation of supramolecular activation clusters in T cells. *Nature* **395**, 82–86 (1998).
5. Grakoui, A. *et al.* The immunological synapse: a molecular machine controlling T cell activation. *Science* 285, 221–227 (1999).

6. Iezzi, G., Karjalainen, K. & Lanzavecchia, A. The duration of antigenic stimulation determines the fate of naive and effector T cells. *Immunity* **8**, 89–95 (1998).
7. Narumiya, S., Sugimoto, Y. & Ushikubi, F. Prostanoid receptors: structures, properties, and functions. *Physiol. Rev.* **79**, 1193–1226 (1999).
8. FitzGerald, G.A., Healy, C. & Daugherty, J. Thromboxane $A_2$ biosynthesis in human disease. *Fed. Proc.* **46**, 154–158 (1987).
9. Namba, T. *et al.* Mouse thromboxane $A_2$ receptor: cDNA cloning, expression and northern blot analysis. *Biochem. Biophys. Res. Commun.* **184**, 1197–1203 (1992).
10. Ushikubi, F. *et al.* Thromboxane $A_2$ receptor is highly expressed in mouse immature thymocytes and mediates DNA fragmentation and apoptosis. *J. Exp. Med.* **178**, 1825–1830 (1993).
11. Murota, S., Kawamura, M. & Morita, I. Transformation of arachidonic acid into thromboxane $B_2$ by the homogenates of activated macrophages. *Biochim. Biophys. Acta* **528**, 507–511 (1978).
12. Nüsing, R. & Ullrich, V. Immunoquantitation of thromboxane synthase in human tissues. *Eicosanoids* **3**, 175–180 (1990).
13. Tone, Y. *et al.* Abundant expression of thromboxane synthase in rat macrophages. *FEBS Lett.* **340**, 241–244 (1994).
14. Brune, K., Glatt, M., Kalin, H. & Peskar, B.A. Pharmacological control of prostaglandin and thromboxane release from macrophages. *Nature* **274**, 261–263 (1978).
15. Nüsing, R. *et al.* Localization of thromboxane synthase in human tissues by monoclonal antibody Tü 300. *Virchows Arch. A Pathol. Anat. Histopathol.* **421**, 249–254 (1992).
16. Morinelli, T.A. *et al.* Characterization of an $^{125}$I-labeled thromboxane $A_2$/prostaglandin $H_2$ receptor agonist. *J. Pharmacol. Exp. Ther.* **251**, 557–562 (1989).
17. Thomas, D.W. *et al.* Coagulation defects and altered hemodynamic responses in mice lacking receptors for thromboxane $A_2$. *J. Clin. Invest.* **102**, 1994–2001(1998).
18. Kouzan, S. *et al.* Stimulation of arachidonic acid metabolism by adherence of alveolar macrophages to a plastic substrate. Modulation by fetal bovine serum. *Am. Rev. Respir. Dis.* **137**, 38–43 (1988).
19. Truneh, A., Albert, F., Golstein, P. & Schmitt-Verhulst, A.M. Early steps of lymphocyte activation bypassed by synergy between calcium ionophores and phorbol ester. *Nature* **313**, 318–320 (1985).
20. Al-Alwan, M., Rowden, G., Lee, T.D.G. & West, K.A. The dendritic cell cytoskeleton is critical for the formation of the immunological synapse. *J. Immunol.* **166**, 1452–1456 (2001).
21. Lanzavecchia, A. & Sallusto, F. Antigen decoding by T lymphocytes: from synapses to fate determination. *Nat. Immunol.* **2**, 487–492 (2001).
22. Zheng, L. *et al.* Induction of apoptosis in mature T cells by tumour necrosis factor. *Nature* **377**, 348–351 (1995).
23. Inaba, K. & Steinman, R.M. Monoclonal antibodies to LFA-1 and to CD4 inhibit the mixed leukocyte reaction after the antigen-dependent clustering of dendritic cells and T lymphocytes. *J. Exp. Med.* **165**, 1403–1417 (1987).
24. Katagiri, K., Hattori, M., Minato, N. & Kinashi, T. Rap1 functions as a key regulator of T-cell and antigen-presenting cell interactions and modulates T-cell responses. *Mol. Cell. Biol.* **22**,1001–1015 (2002).
25. Bromley, S.K., Peterson, D.A., Gunn, M.D. & Dustin, M.L. Hierarchy of chemokine receptor and TCR signals regulating T cell migration and proliferation. *J. Immunol.* **165**,15–19 (2000).
26. Krummel, M.F. & Davis, M.M. Dynamics of the immunological synapse: finding, establishing and solidifying a connection. *Curr. Opin. Immunol.* **14**, 66–74 (2002).
27. Allen, P.M. & Unanue, E.R. Differential requirements for antigen processing by macrophages for lysozyme-specific T cell hybridomas. *J. Immunol.* **132**, 1077–1079 (1984).
28. Lombard-Platlet, S., Bertolino, P., Deng, H., Gerlier, D. & Rabourdin-Combe, C. Inhibition of chloroquine of the class II major histocompatibility complex-restricted presentation of endogenous antigens varies according to the cellular origin of the antigen-presenting cells, the nature of the T-cell epitope, and the responding T cell. *Immunology* **80**, 566–573 (1993).
29. Kurimoto, I. *et al.* Studies of contact hypersensitivity induction in mice with optimal sensitizing doses of hapten. *J. Invest. Dermatol.* **101**, 132–136 (1993).
30. Hanasaki, K. & Arita, H. Characterization of a new compound, S-145, as a specific $TXA_2$ receptor antagonist in platelets. *Thromb. Res.* **50**, 365–376 (1988).
31. Nagai, H. *et al.* Different role of IL-4 in the onset of hapten-induced contact hypersensitivity in BALB/c and C57BL/6 mice. *Br. J. Pharmacol.* **129**, 299–306 (2000).
32. Ogletree, M.L. Overview of physiological and pathophysiological effects of thromboxane $A_2$. *Fed Proc.* **46**, 133–138 (1987).
33. Daniel, T.O., Liu, H., Morrow, J.D., Crews, B.C. & Marnett, L.J. Thromboxane $A_2$ is a mediator of cyclooxygenase-2-dependent endothelial migration and angiogenesis. *Cancer Res.* **59**, 4574–4577 (1999).
34. Dustin, M.L. & Cooper, J.A. The immunological synapse and the actin cytoskeleton: molecular hardware for T cell signaling. *Nat. Immunol.* **1**, 23–29 (2000).
35. Dustin, M.L. *et al.* A novel adaptor protein orchestrates receptor patterning and cytoskeletal polarity in T-cell contacts. *Cell* **94**, 667–677 (1998).
36. Wülfing, C., Sjaastad, M.D. & Davis, M.M. Visualizing the dynamics of T cell activation: Intracellular adhesion molecule 1 migrates rapidly to the T cell/B cell interface and acts to sustain calcium levels. *Proc. Natl. Acad. Sci. USA* **95**, 6302–6307 (1998).
37. Bleijs, D.A., Geijtenbeek, T.B., Figdor, C.G. & van Kooyk, Y. DC-SIGN and LFA-1: a battle for ligand. *Trends. Immunol.* **22**, 457–463 (2001).
38. Yong, K. & Khwaja, A. Leucocyte cellular adhesion molecules. *Blood Rev.* **4**, 211–225 (1990).
39. Cifone, M.G. *et al.* Effect of hrTNF $\alpha$ on $TxB_2$ release by macrophages. *Int. J. Tissue React.* **9**, 51–53 (1987).
40. Conti, P. *et al.* In vitro enhanced thromboxane $B_2$ release by polymorphonuclear leukocytes and macrophages after treatment with human recombinant interleukin 1. *Prostaglandins* **32**, 111–115 (1986).
41. Goldstein, I.M. *et al.* Thromboxane generation by human peripheral blood polymorphonuclear leukocytes. *J. Exp. Med.* **148**, 787–792 (1978).
42. Sun, L.K., Wahl, P., Bilic, G. & Wuthrich, R.P. CD44-mediated cyclooxygenase-2 expression and thromboxane $A_2$ production in RAW 264.7 macrophages. *Inflamm. Res.* **50**, 496–499 (2001).
43. Wrenger, S. *et al.* Down-regulation of T cell activation following inhibition of dipeptidyl peptidase IV/CD26 by the N-terminal part of the thromboxane $A_2$ receptor. *J. Biol. Chem.* **275**, 22180–22186 (2000).
44. Mitsuhashi, M. *et al.* Necessity of thromboxane $A_2$ for initiation of platelet-mediated contact sensitivity: dual activation of platelets and vascular endothelial cells. *J. Immunol.* **166**, 617–623 (2001).
45. Kabashima, K. *et al.* The prostaglandin receptor EP4 suppresses colitis, mucosal damage and CD4 cell activation in the gut. *J. Clin. Invest.* **109**, 883–893 (2002).
46. Kweon, M.N., Yamamoto, M., Kajiki, M., Takahashi, I. & Kiyono, H. Systemically derived large intestinal $CD4^+T_H2$ cells play a central role in STAT6-mediated allergic diarrhea. *J. Clin. Invest.* **106**, 199–206 (2000).