

Review

Novel concept of iSALT (inducible skin-associated lymphoid tissue) in the elicitation of allergic contact dermatitis

By Tetsuya HONDA^{*1,†} and Kenji KABASHIMA^{*1,†}

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Abstract: Allergic contact dermatitis (ACD) is one of the most common inflammatory skin diseases, which is classified as a delayed-type hypersensitivity immune response. The development of ACD is divided into two phases: sensitization and elicitation. In the sensitization phase, antigen-specific effector T cells are induced in the draining lymph nodes by antigen-captured cutaneous dendritic cells (DCs) that migrate from the skin. In the elicitation phase, the effector T cells are activated in the skin by antigen-captured cutaneous DCs and produce various chemical mediators, which create antigen-specific inflammation. In this review, we discuss the recent advancements in the immunological mechanisms of ACD, focusing on the mechanisms in the elicitation phase. The observations of elicitation of CHS lead to the emerging novel concept of iSALT (inducible skin-associated lymphoid tissue).

Keywords: allergic contact dermatitis, contact hypersensitivity, dendritic cells, T cells, iSALT

Introduction

Allergic contact dermatitis (ACD), such as metal allergy and plant allergy, is a major occupational skin disease that affects approximately 15 to 20% of the general population all over the world.¹⁾ ACD is generally induced by small compounds called haptens (less than 500 daltons of molecular weight). Although haptens do not possess antigenicity, they bind to self-carrier protein in the skin, and the hapten-self complex works as an antigen.^{2)–4)} This complex is captured by cutaneous dendritic cells (DCs), which migrate to the draining lymph nodes (dLNs) and present the antigen to naïve T cells. Then, the naïve T cells proliferate and differentiate to several T cell subsets, such as CD4⁺ helper T (Th)1 cells and CD8⁺ cytotoxic T (Tc)1 cells. This

priming phase is called the sensitization phase (Fig. 1). When the same hapten enters the skin, the antigen-specific Th1/Tc1 cells are activated by the antigen-captured cutaneous DCs. The activated Th1/Tc1 cells produce cytokines such as IFN- γ , which stimulates neighboring cells such as keratinocytes, and provokes inflammation that peaks around 24 to 48 h after the hapten exposure. This phase is called the elicitation phase (Fig. 1).

Since ACD is a prototype of the cutaneous immune response, and the murine model of ACD, which is called contact hypersensitivity (CHS),⁵⁾ is easily induced, a number of studies have been performed to determine its mechanisms, especially in the sensitization phase. For example, extensive studies have been performed for the cutaneous DC subset analysis that induces Th1/Tc1 differentiation in the sensitization phase. In contrast, relatively few studies have been performed on the DC subset responsible in the elicitation phase. This may be partly because of the complex cell-cell interactions in the elicitation phase. However, with the introduction of novel techniques such as multi-photon microscopy for live imaging, the complex mechanisms of the elicitation phase have recently been gradually revealed.

^{*1} Department of Dermatology, Kyoto University Graduate School of Medicine, Kyoto, Japan.

[†] Correspondence should be addressed: K. Kabashima and T. Honda, Department of Dermatology, Kyoto University Graduate School of Medicine, 54 Shogoin-Kawara, Sakyo, Kyoto 606-8507, Japan (e-mail: kaba@kuhp.kyoto-u.ac.jp (KK) or hontetsu@kuhp.kyoto-u.ac.jp (TH)).

Abbreviations: DC: dendritic cell; ACD: allergic contact dermatitis; CHS: contact hypersensitivity; iSALT: inducible skin-associated lymphoid tissue; LC: Langerhans cell.

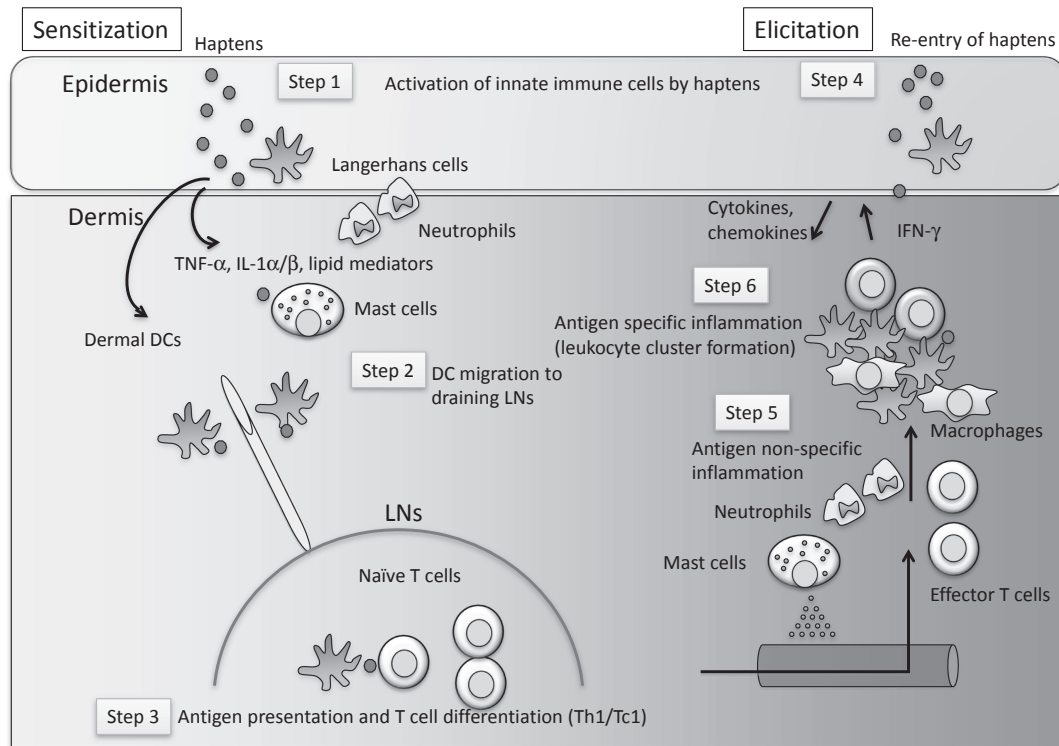


Fig. 1. Overview of the immunological mechanisms of CHS. Step 1. Haptens activate innate immune cells (e.g., keratinocytes, mast cells) and induce the production of various chemical mediators. Step 2. Antigen-captured activated DCs migrate to the dLNs. Step 3. Migrated DCs present the antigen to naïve T cells, which mainly differentiate to Th1 and Tc1 cells. Step 4. Haptens cause subtle inflammation by activating innate immune cells, and recruit neutrophils. Step 5. Leukocytes, including antigen-specific effector T cells, are recruited. Step 6. The antigen-specific effector T cells are then activated in the skin by antigen-captured dermal DCs, which create antigen specific inflammation. Activation of effector T cells mainly occurs in leukocyte clusters.

In this review, we will introduce the updated mechanisms of ACD, as revealed by using mouse CHS, mainly focusing on the mechanisms in the elicitation phase.

1. Induction of antigen-specific T cells

Although various kinds of cells are involved in the development of CHS, the most important cells for antigen specific inflammation are T cells. Both Th cells and Tc cells play important roles in the development of CHS.^{6)–10)} Th/Tc cells are further divided into several kinds of Th/Tc cell subsets, such as Th1/Tc1, Th2, and Th17/Tc17 cells, depending on the pattern of its cytokine production. Among the Th/Tc cell subsets, Th1/Tc1 cells, which produce IFN- γ , are the most important cell subsets that induce the elicitation reaction,^{5),11)} although the involvement of other Th/Tc cell subsets such as Th2 or Th17 have been reported.^{9),10)} For many years, the cutaneous DC subsets responsible for inducing Th1/Tc1 have been a matter of debate.

However, recent developments in the DC-subset-specific depletion system have gradually answered these questions. First, we summarize the recent findings on the role of each cutaneous DC subset in the sensitization phase.

In the skin in the steady state, there are at least three DC subsets; Langerhans cells (LCs), CD103⁺ dermal DCs, and CD103[−] dermal DCs.^{12)–14)} LCs exist in the epidermal layer, and are the most abundant DCs in the skin. CD103[−] dermal DCs occupy most of the dermal DCs (around 80% of dermal DCs) while CD103⁺ dermal DCs occupy around 10% of dermal DCs.^{12)–14)}

LCs have long been assumed to be the DCs responsible for inducing Th1/Tc1 cells in CHS, because of their abundance in the skin, the easy accessibility to haptens, and the strong antigen-presentation ability *in vitro*. However, recent studies have revealed that depletion of LCs during the sensitization phase does not impair CHS responses, while depletion of CD103⁺ dermal DCs causes

significantly impaired CHS responses.^{12),15),16)} Therefore, the general current understanding is that CD103⁺ dermal DCs are the most important DC subset that mediates sensitization in CHS. However, CD103⁺ dermal DCs may not be the essential DCs for sensitization, because the impairment in CHS responses caused by the depletion of CD103⁺ dermal DCs is partial, and Batf^{-/-} mouse, which lacks CD103⁺ dermal DCs constitutively, exhibits normal CHS responses.¹⁷⁾ In addition, the redundant roles of CD103⁺ dermal DCs and LCs in sensitization^{18),19)} and the importance of CD103⁻ dermal DCs in sensitization have been reported.^{18),20)} Therefore, although CD103⁺ dermal DCs are proposed as the DCs responsible for mediating sensitization in CHS, the current data suggest that each DC subset has the ability to mediate sensitization in a context dependent manner.

In humans, two dermal DC subsets are also identified, depending on the expression pattern of CD1a, CD1c, and CD141 (CD1a⁺CD1c⁺ dermal DCs and CD1a⁺CD141⁺ dermal DCs).²¹⁾ CD1a⁺CD141⁺ dermal DCs are proposed to be identical to mouse CD103⁺ dermal DCs.²¹⁾ These DCs may mediate sensitization in human ACD.

2. Mechanisms of the effector T cell activation in the elicitation phase

Effector T cells are recruited to and retained in inflammatory skin with limited dependency on their antigen specificity.²²⁾ Therefore, effector Th1/Tc1 cells infiltrate the skin following the subtle inflammation induced by haptens. Indeed, initial neutrophil infiltration is necessary for subsequent effector T cell infiltration in the elicitation phase.^{23)–26)} When the concentration of haptens is not high enough to provoke this antigen non-specific inflammation, no CHS response occurs.²⁷⁾ Haptens induce skin inflammation directly by causing cellular stress and indirectly by activating toll-like receptors (TLRs) and NOD-like receptors (NLRs). The infiltrated effector T cells are then activated by antigen-captured cutaneous DCs, and produce cytokines, which provoke antigen-specific inflammation.

Although the detailed mechanisms by which haptens induce skin inflammation remain unknown, recent reports indicate that activation of TLRs and NLRs in innate immune cells is an important mechanism of hapten-induced inflammation.^{11),28),29)} Haptens induce cellular stresses and damages that lead to the production of reactive oxygen species (ROS).^{30)–35)} ROS degrade the extracellular matrix

(such as hyaluronic acid), and generate low-molecular-weight hyaluronic acid,²⁸⁾ which stimulates TLR2 and TLR4 on the surrounding cells, such as DCs, keratinocytes, and mast cells, all of which express TLR2 and TLR4.^{36)–40)} Stimulation of TLR2 and TLR4 eventually leads to the activation of nuclear factor κ B (NF- κ B) and mitogen-activated protein kinases (MAPKs), and induces the expression of various pro-inflammatory cytokines and chemokines, which drive both DC migration to dLNs and the inflammatory cell infiltration into the skin. Haptens also induce the release of adenosine triphosphate (ATP).^{29),30)} ATP stimulates purinergic receptor P2X7, which activates NOD-, leucine rich region repeat (LRR)-, and pyrin domain-containing 3 (NLRP3).⁴¹⁾ Activation of NLRP3 in keratinocytes leads to the release of IL-1 β and IL-18, which also drives skin inflammation.^{42)–44)} Haptens also increase vascular permeability by inducing histamine release from mast cells.⁴⁵⁾ Overall, haptens cause innate immune cell activation and induce the initial neutrophil infiltration to recruit effector T cells in the skin (Fig. 2).

3. Antigen-specific inflammation: Leukocyte cluster formation as an essential structure for effector T cell activation in the skin

Following the hapten-induced antigen non-specific inflammation, T cell-mediated antigen-specific inflammation is initiated. When T cells infiltrate into the skin, they induce a stable interaction with antigen-bearing cutaneous DCs and produce cytokines.^{22),46)} Dermal DCs play essential roles in the effector T cell activation,⁴⁷⁾ although the dermal DC subset responsible for the antigen presentation in the elicitation phase remains unclear. Cytokines produced by activated T cells then stimulate skin-resident cells, which leads to further recruitment of T cells and amplification of the inflammation.

We recently discovered that dermal leukocytes form a cluster structure after hapten application, and that this structure is essential for efficient effector T cell activation in the skin.⁴⁷⁾ In skin in the steady state, cutaneous DCs are distributed randomly and exhibit active motility. However, after hapten application, DCs exhibit cluster formation around postcapillary venules. Effector T cells also accumulate in the cluster. Depletion of macrophages completely abrogates the DC cluster formation and is accompanied by impaired effector T cell activation in the skin. Blockade of IL-1 α or CXCL2 impair the leukocyte cluster formation and effector T cell

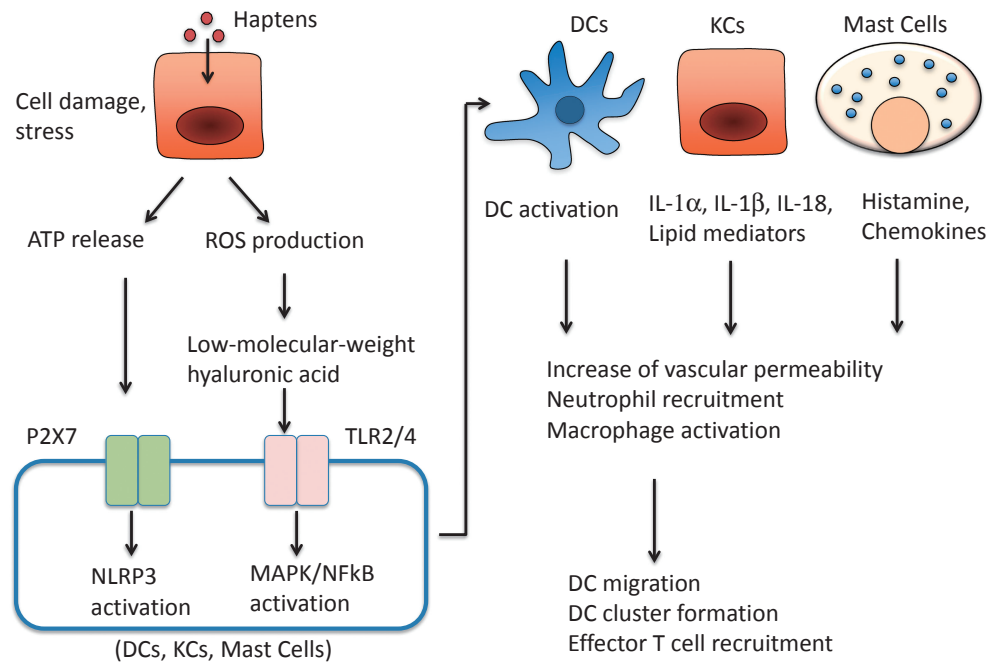


Fig. 2. A schematic view of hapten-induced inflammation. Haptens induce ATP release and ROS production from several skin cells, such as keratinocytes. NLRP3 activation and/or MAPK/NF- κ B activation are induced via P2X7 signaling and TLR2/4 signaling, which are stimulated by ATP and low-molecular-weight hyaluronic acid, respectively. KCs and mast cells produce various chemical mediators, and drive skin inflammation. This first round of inflammation is essential for the subsequent DC migration in the sensitization phase and effector T cell infiltration in the elicitation phase.

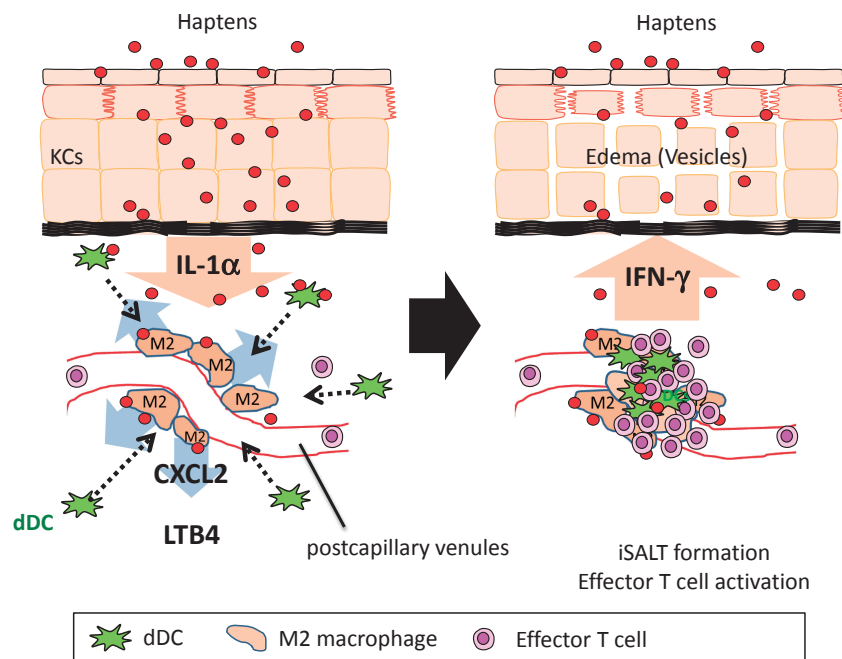


Fig. 3. A schematic view of iSALT formation. Haptens induce IL-1 α production from keratinocytes (KCs), which stimulates M2-type macrophages located around postcapillary venules. The stimulated macrophages then produce CXCL2, which accumulate dermal DCs. LTB4 also plays an important role in DC accumulation by increasing DC motility. Effector T cells are activated within the DC clusters (iSALT), and produce cytokines.

activation. Keratinocytes are the main producers of IL-1 α . Among the two types of macrophage subsets (classically activated (M1)- and alternatively activated (M2)-type macrophages), M2-type macrophages have significantly higher expression of IL-1 receptor, and produce CXCL2 upon IL-1 α stimulation.⁴⁷⁾ These results indicate that the leukocyte cluster is an essential structure for efficient T cell activation in the skin, and that the IL-1 α -CXCL2 axis via M2 macrophages mediates the cluster formation⁴⁷⁾ (Fig. 3). In addition to CXCL2, leukotriene B4 (LTB4), a lipid mediator, mediates the leukocyte cluster formation by promoting DC motility through Cdc42 and Rac activation.⁴⁸⁾

This leukocyte cluster formation may also be important for the development of human ACD, because the leukocyte clusters are observed in human ACD, and edema of the epidermal layers (which is an indicator of effector T cell activation) occurs above the leukocyte clusters.⁴⁷⁾ These findings suggest the importance of the leukocyte cluster in human ACD.

Since the 1980's, the concept of skin-associated lymphoid tissues (SALT) was proposed as the structure for T cell activation in the skin.⁴⁹⁾ However, the existence and significance of SALT has never been proved. The leukocyte clusters we observed may correspond with the old concept of SALT. However, we propose that the leukocyte clusters are inducible SALT (iSALT), since they are induced in the inflammatory state and do not exist in the steady state.⁵⁰⁾

4. Regulation of elicitation

4-1. Regulatory T cells. Regulatory T cells (Tregs) are a T cell subset that exerts a potent immunosuppressive effect to inhibit auto-reactive T cell activation.⁵¹⁾ Tregs also exert regulatory roles in various inflammatory diseases, including CHS.⁵²⁾ The number of Tregs in the skin significantly increases during the skin inflammation process,⁵³⁾ and depletion of Tregs in the elicitation phase as well as the sensitization phase causes enhanced and prolonged inflammatory responses,^{53),54)} indicating that Tregs play crucial roles in the regulation of elicitation. Although the detailed mechanisms of the suppression remain unknown, the inhibition of leukocyte influx into the skin via IL-10 or CD39/73 in Tregs is proposed as one of the regulatory mechanisms.^{55)–57)} Inhibition of stable DC-T cell interaction may be another possible regulatory mechanism by Treg in CHS.⁵⁸⁾ In addition, skin

Tregs may exert a suppressive function by inhibiting antigen-specific T cell proliferation in the dLNs by recirculation.⁵³⁾

Using a new cell labeling system with the photo-convertible protein Kaede, we succeeded in tracking T cell migration after their infiltration to the skin. Interestingly, the skin-derived Tregs exhibited an activated phenotype with high expression of cytotoxic T lymphocytes antigen-4 and IL-10, and had much more potent suppressive activities than resident Tregs in the dLNs, suggesting that skin Tregs exert their potent immunosuppressive activity not only in the skin but also in the dLNs by circulating in the body.⁵³⁾

4-2. Langerhans cell as a possible regulator of sensitization and elicitation. As mentioned earlier, the theory that LCs work as initiators of CHS is now challenged. Rather, recent reports suggest that LCs work as regulators of sensitization and elicitation. In a constitutive or inducible LC-depletion system, LC depletion in the sensitization phase causes enhanced CHS responses.^{59),60)} In that system, LCs exert regulatory functions via cognate CD4 interaction and the production of IL-10.⁵⁹⁾ In a dinitrothiocyanobenzene-induced cutaneous immune tolerance model, LCs induce tolerance by activating Tregs.⁶¹⁾ It is also reported that LCs induce Tregs in an ultraviolet (UV)-induced immunosuppression model as well as a skin graft-induced immunosuppression model.^{62),63)}

Thus, LCs may play regulatory roles in the sensitization of CHS, at least under certain conditions. In the elicitation phase, the role of LCs is less clear. However, several reports also suggest the regulatory role of LCs in the elicitation phase. In an old study that depleted skin DCs during the elicitation phase by topical steroid, mice exhibited enhanced CHS responses,⁶⁴⁾ suggesting the existence of DC subsets that play a regulatory function in the elicitation phase. Considering the stimulatory roles of dermal DCs in the elicitation phase,⁴⁷⁾ the regulatory roles may be executed by LCs. In human studies, it is reported that LCs play essential roles in the maintenance of Tregs in skin in the steady state.⁶⁵⁾ LCs may inhibit effector CD4 T cell activation in patients with nickel allergy via expression of programmed-death ligand-1.⁶⁶⁾ Overall, although the function of LCs in CHS remains controversial, LCs may work as regulators of CHS in both sensitization and elicitation phases. Clarification of the mechanisms would be of great interest and benefit from both basic science and clinical points of view.

Conclusion and discussion

By introducing new technologies for the analysis of CHS, the immunological mechanisms of ACD have been getting clearer. One of the remaining points to be clarified is whether findings in mouse CHS are relevant to human ACD. Another interesting question is the role of iSALT in inflammatory skin diseases other than ACD. In fact, in psoriasis, a common inflammatory skin disease, an iSALT-like structure has been reported in the skin lesions.⁶⁷⁾ iSALT may serve as an important structure to provoke inflammation in psoriasis as well. In addition, although T cells and DCs are the major players in the development of ACD, the roles of innate immune cells, such as mast cells^{45),68)} and macrophages, neutrophils,⁶⁹⁾ and natural killer cells⁷⁰⁾ in acquired immunity are also attracting attention. The crosstalk between innate immune cells and acquired immune cells would be an important mechanism to create antigen-specific immune responses. Evaluation of these points may lead to a breakthrough in the understanding of the immunological mechanisms of various cutaneous immune responses, including ACD.

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Profile

Kenji Kabashima was born in Takayama City in 1970, and brought up in northern part of Kyushu in Japan. He graduated from Kyoto University in 1996. He trained in Medicine/Dermatology at the United Naval Hospital, Kyoto University Hospital, and University of Washington Medical Center. He started research on lipid mediators in immunology at Kyoto University, which led to a Ph.D. (Prof. Shuh Narumiya) in 2003. That year, he was appointed as assistant professor in Dermatology at Kyoto University (Prof. Yoshiki Miyachi), and researched on dendritic cell homeostasis and plasma cell mobilization at University of California San Francisco (Prof. Jason Cyster). In 2005, he moved to University of Occupational and Environmental Health as an associate professor (Prof. Yoshiki Tokura). He was assigned as an associate professor in 2008 and became a professor and chairman in Dermatology at Kyoto University in 2015. He has been researching on the translational medicine and mechanism of inflammatory skin diseases by gene-targeted mice and visualization of the skin. For his accomplishment, he received the JSPS prize and PhARF award (from Europe). He is currently an executive board member of the Japanese Dermatological Association, International League of Dermatological Societies, and International Eczema Council.

