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4. 脂質メディエーターと皮膚免疫・アレルギー疾患

椛島健治

生体が刺激にさらされると脂質メディエーターは細胞膜より放出され，ホメオスタシスの維持や病態形成に重要な役割を果たしていると考えられてきたがその詳細は長年不明であった．近年これらの合成酵素，受容体の遺伝子改変マウスや選択的薬物の開発により，脂質メディエーターの皮膚免疫・アレルギー疾患における生理的病態的役割の解明とその臨床応用が目覚しく進んでいる．本稿では最新の知見を交えて皮膚免疫・アレルギー疾患における脂質メディエーターの役割を解説する．

はじめに

生体が刺激を受けると，細胞膜リン脂質よりアラキドン酸が放出され，シクロオキシゲナーゼ（COX）と各合成酵素によりプロスタノイドと呼ばれるプロスタグランジン（PG）D_2，PGE_2，$PGF_{2\alpha}$，PGI_2やトロンボキサン（TX）A_2に変換される．また，アラキドン酸は，5-リポキシゲナーゼ（5-LO），ロイコトリエン（LT）A_4加水分解酵素，LTC_4合成酵素などによりLTB_4やシステニル（cys）LTであるLTC_4，LTD_4，LTE_4にも変換される．さらに，スフィンゴシン1-リン酸（S1P）やリポキシン，血小板活性化因子なども脂質メディエーターの1つである．これらは一般に，近傍に存在する標的細胞上のGタンパク質結合型受容体を介して生理的役割を発揮する（**表1**）．脂質メディエーターの受容体はさまざまな皮膚免疫担当細胞に発現しており，近年これらの合成酵素や受容体の遺伝子改変マウスや選択的アゴニスト，アンタゴニストの開発により，免疫・アレルギーにおける生理的病態的役割が明らかになりつつある（**表2**）[1]．

[キーワード&略語]
アトピー性皮膚炎，接触皮膚炎，アスピリン不耐症，T細胞，樹状細胞

AD：atopic dermatitis（アトピー性皮膚炎）
COX：cyclooxygenase（シクロオキシゲナーゼ）
cys：cysteinyl（システニル）
NSAID：non-steroidal anti-inflammatory drug（非ステロイド系抗炎症薬）
PG：prostaglandin（プロスタグランジン）
S1P：sphingosine 1-phosphate（スフィンゴシン1-リン酸）
TNF-α：tumor necrosis factor α（腫瘍壊死因子）
TX：thromboxane（トロンボキサン）

1 NSAIDと皮膚疾患

これまでプロスタノイドの役割は，COX阻害薬である非ステロイド系抗炎症薬（non-steroidal anti-inflammatory drug：NSAID）の効果をもとに推測さ

Lipid mediators in cutaneous immune and allergic diseases
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表1　皮膚免疫関連細胞における脂質メディエーター受容体の発現

脂質メディエーター	PGD_2		PGE_2				$PGF_{2\alpha}$	PGI_2	TXA_2	cys LT		LTB_4	
脂質メディエーターの受容体	DP	CRTH2	EP1	EP2	EP3	EP4	FP	IP	TP	cys LT1	cys LT2	BLT1	BLT2
樹状細胞	m, h			m, h		m, h						m	m, h
T cell		m (Th2)	m	m, h	m, h	m, h		m	m	h		m (Effector T)	
B cell			m, h	m, h	m, h	m, h						m, h	
好酸球		m		h		h			h	m, h	h	m	
肥満細胞・好塩基球	m	m	m	m	m	m				h	h	m	
好中球	h			m, h		m				h		m, h	
血管			m, h	m, h	m, h	m, h		m, h	m, h				

m：mouse，h：human，ここに記した以外にも多くの受容体が各細胞で発現されることが報告されている

表2　脂質メディエーター受容体の免疫・アレルギーにおける役割のまとめ

脂質メディエーター	その受容体	これまで報告された生理的作用
PGD_2	DP	卵白アルブミン誘発アレルギー喘息反応の亢進（マウス）
	CRTH2	Th2細胞や好酸球・好中球の化学遊走作用(マウス)
PGE_2	EP1	Th1への分化誘導（マウス）
	EP2	リンパ球混合試験反応におけるT細胞反応の抑制（マウス，ヒト）
	EP3	発熱物質に対する発熱反応形成（マウス）
	EP4	Th1/Th17への分化誘導，樹状細胞機能の亢進による接触過敏反応形成（マウス）
$PGF_{2\alpha}$	FP	(−)
PGI_2	IP	マウス炎症性腫脹，酢酸ライジング反応，卵白アルブミン誘発アレルギー喘息反応の抑制，Th1への分化誘導（マウス）
TXA_2	TP	T細胞と樹状細胞の免疫シナプスの阻害，アトピー性皮膚炎の抑制（マウス）
LTB4	BLT1	メモリーT細胞の遊走，樹状細胞のTh1誘導（マウス）
	BLT2	表皮角化細胞の遊走
	責任受容体不明	好中球の遊走や活性化→尋常性乾癬・尋常性ざ瘡に関与 膨疹と紅斑反応の誘発（ヒト）
cys LT（LTC_4, D_4, E_4）	cys LT1	好酸球の遊走・炎症局所への浸潤（ヒト）→遅発型反応（late phase reaction)の形成，血管拡張・透過性の亢進，マクロファージ・好酸球・肥満細胞の活性化，受身皮膚アナフィラキシー反応（マウス）
	cys LT2	IL-8の産生を介した好中球の浸潤，血管内皮細胞の活性化
	責任受容体不明	ランゲルハンス細胞の所属リンパ節への遊走（マウス）
PAF	PAFR	卵白アルブミン誘発アレルギー喘息反応誘発（マウス）
S1P	S1P1	胸腺やリンパ節からのリンパ球の遊出（マウス，ヒト）

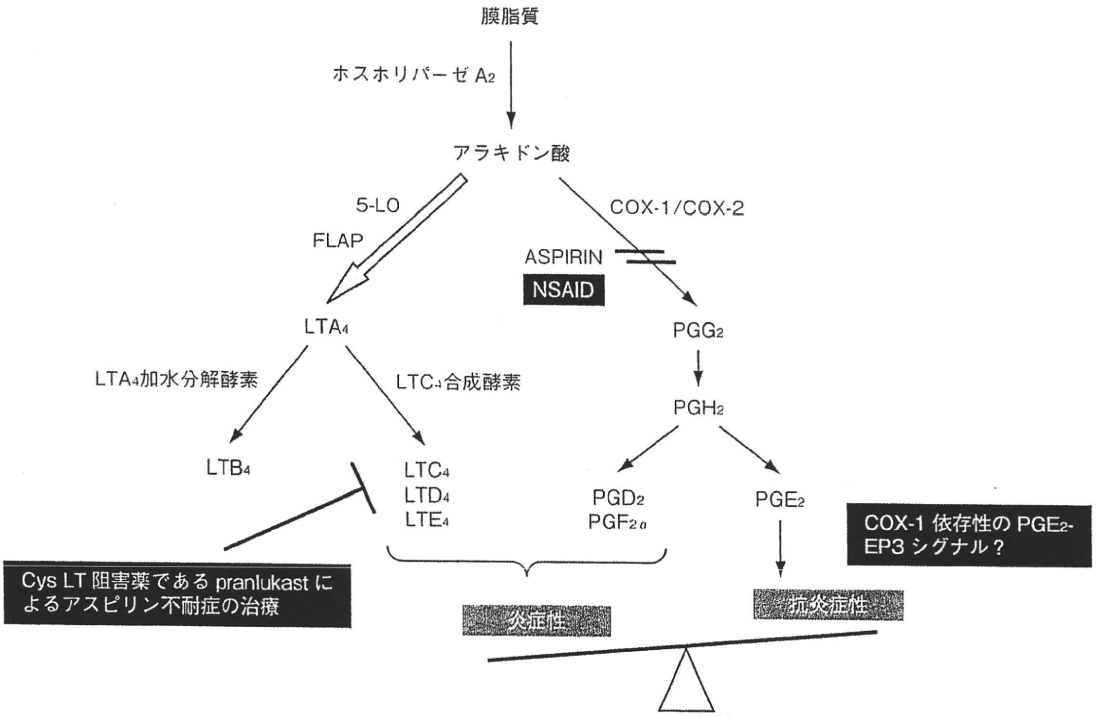

図1　アスピリン不耐症の発症機序仮説

COX-2阻害薬ではアスピリン不耐症が発症しにくいことより，COX-1依存型のプロスタノイドの減少，あるいは，NSAIDによりアラキドン酸代謝系がLT産生系に傾くことが発症機序として想定されている．近年EP3欠損マウスでアレルギー喘息モデルの増悪が認められ[2]，アスピリン不耐症におけるPGE_2-EP3シグナルの関与が示唆される

れてきた．例えば，ざ瘡において，NSAIDの1つであるイブプロフェンピコノールは抗炎症作用を有し，*Propionibacterium acnes*菌による白血球遊走能や細菌性リパーゼに対する活性阻止作用を有することが報告されている．そのため，特に毛孔性の丘疹，膿疱を有するざ瘡に対して効果がある．また，好酸球性膿疱性毛包炎においてインドメタシン内服や外用が有効な症例を多く経験する．ところが，アレルギー性皮膚疾患においては効果が一定せず，しかもかぶれを起こしやすいこと，さらにステロイド外用剤の出現により，その意義は薄れ，プロスタノイドの皮膚における重要性は省みられなくなった．

一方，アスピリン不耐症※1がCOX-2選択的阻害剤では起こりにくいという報告があり，COX-1依存型のプロスタノイドの減少が気道の狭窄や肥満細胞の活性を促進するという機序が考えられている．近年EP3欠損マウスでアレルギー喘息モデルの増悪が認められ[2]，アスピリン不耐症におけるPGE_2-EP3シグナルの関与が示唆される．また，NSAIDにより脂質代謝系がLT産生系に傾くためにアスピリン不耐症が起こるという考え方もあり，それに合致するかのように，アスピリン不耐症におけるcys LT阻害薬の有効性が報告されている（図1）．

※1　アスピリン不耐症

アスピリンをはじめとするNSAIDにより蕁麻疹，鼻炎，喘息，時にアナフィラキシーショック様の症状を呈する疾患である．特異的IgE抗体を認めないこと，皮内テストが陰性であること，COX阻害作用の強さと症状の程度が相関することから，I型アレルギー反応と異なるNSAIDの薬理学的作用による非アレルギー性疾患と考えられている．

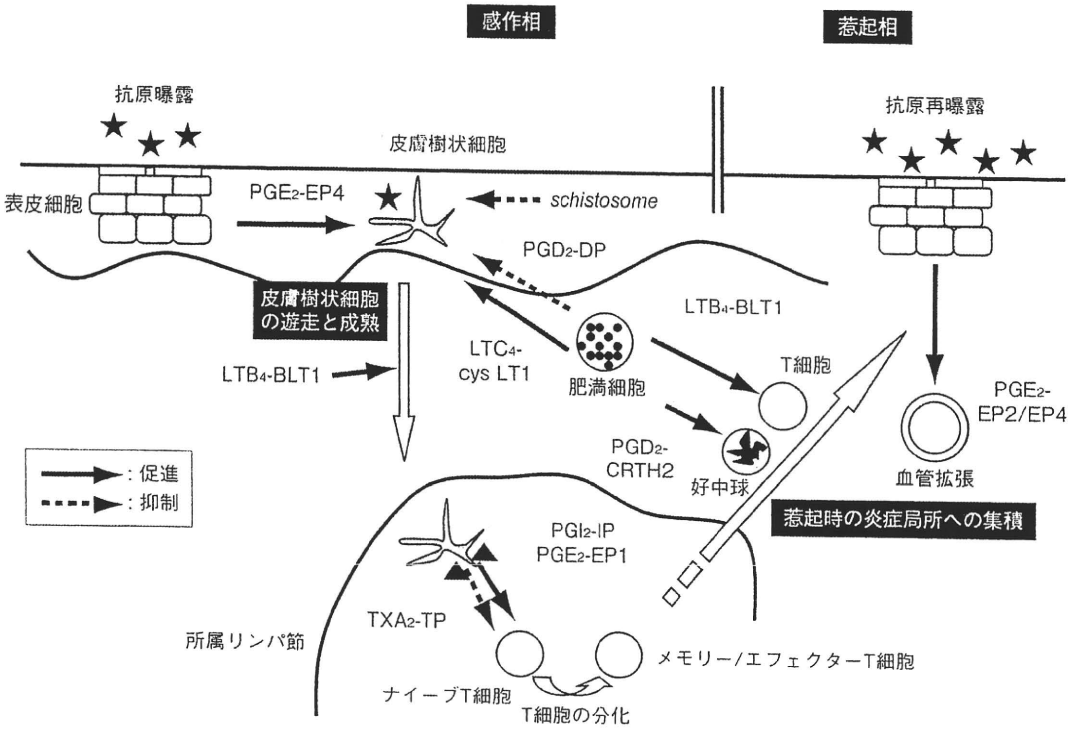

図2　接触皮膚炎における脂質メディエーターの役割

皮膚への抗原曝露に伴い，炎症性のメディエーターが表皮細胞から放出され，皮膚樹状細胞は活性化し抗原を取り込み成熟しながら所属リンパ節へ遊走し，T細胞へ抗原提示を行う．この際表皮細胞，肥満細胞，皮膚樹状細胞などからさまざまな脂質メディエーターが産生され，図に示すような多彩な役割を果たす．再度同一抗原に皮膚が曝露されると表皮細胞は炎症性のメディエーターやケモカインを産生し，遅延型過敏反応を惹起する．感作相に比べて脂質メディエーターの役割は不明な点が多いが，図に示すような役割を果たすことが明らかにされた

2 接触皮膚炎と脂質メディエーター

1）接触皮膚炎における感作相と惹起相

接触皮膚炎※2では，皮膚がハプテンなどの外来抗原に曝露されると表皮細胞が腫瘍壊死因子（TNF-α）やIL-1βなどの炎症性メディエーターを産生し，皮膚樹状細胞は活性化しながら抗原を取り込み所属リンパ節へと遊走する．リンパ節で皮膚樹状細胞は，ナイーブT細胞をメモリーT細胞へと分化・成熟させ，リンパ節から血液中に移動し，細胞性免疫反応の準備を整える．以上の感作相成立にはおよそ3～5日間要する[3]．

※2　接触皮膚炎

金属アレルギーやピアス皮膚炎などのいわゆる‘かぶれ’のことであり，T細胞を介する遅延型過敏反応の典型で，感作相と惹起相という2相からなる．表皮角化細胞，皮膚樹状細胞，T細胞などのさまざまな皮膚免疫細胞が関与する．

惹起相では，同一抗原曝露に対し，表皮細胞は炎症性メディエーターを産生し局所の血管の接着因子の活性化や，表皮細胞によるケモカイン産生を促し，メモリーT細胞を皮膚の局所へ引き寄せ，IFN-γなどのTh1サイトカインなどを産生し炎症を惹起する（図2）[4]．

2）脂質メディエーターの役割

この接触皮膚炎における脂質メディエーターの役割がマウスを中心とした実験より明らかになりつつある．例えば，抗原曝露により表皮角化細胞から大量に産生されるPGE_2は，EP4受容体を介してマウス皮膚樹状細胞の所属リンパ節への遊走や抗原提示能を亢進させる[5]．活性化した樹状細胞は，PGE_2，TXA_2，PGI_2を産生し，おのおのPGE_2は，T細胞上のEP1やEP4受容体を介してTh1を誘導[6][7]，TXA_2はナイーブT細胞上のTP受容体に作用して樹状細胞とT細胞の相互作用を阻害し免疫反応を抑制[8]，PGI_2はT細胞に作用

してTh1の誘導をcAMP依存的に誘導する[9]．また，経皮感染した*Schistosoma mansoni*は自らPGD$_2$を産生し，樹状細胞上のDPに作用して，遊走能を阻害し自身の感染状態を優位に保つように働く[10]．さらに，惹起時において，マウス好中球などの細胞の皮膚への局所浸潤にCRTH2が関与していることも示された[11]．

LTC$_4$を細胞外に放出するMRP1遺伝子を欠失したマウスでは皮膚樹状細胞の遊走が減弱するが，このマウスにLTC$_4$あるいはLTD$_4$を投与するとその作用が回復する[12]．また，マウス骨髄細胞由来樹状細胞にcys LT1受容体や5-LO，LTC$_4$合成酵素が存在し，cys LT1受容体アンタゴニストにより樹状細胞からのIL-10の産生が抑制され，Th1型反応を誘導するIL-12の産生が亢進する．したがって，樹状細胞自身あるいは肥満細胞が産生するcys LTが，樹状細胞の所属リンパ節への遊走やTh2型反応への誘導を促進していることが示唆される．ロイコトリエン拮抗薬がTh2型炎症反応を抑制するという報告は，上記所見と合致する．

一方，惹起相において活性化肥満細胞あるいは表皮角化細胞の産生するLTB$_4$がBLT1を介してメモリーT細胞を炎症局所へ集積させること，また，樹状細胞上のBLT1を介してIL-12を誘導させ，Th1型免疫反応を亢進させることも近年報告された[13]．以上の結果は脂質メディエーターが接触皮膚炎の感作相と惹起相において，多彩な役割を果たしていることを意味している（**図2**）．

3 アトピー性皮膚炎とプロスタノイド

アトピー性皮膚炎（AD）※3では，病変部や患者血清中のPGE$_2$やTXA$_2$，LTB$_4$，cys LTなどの脂質メディエーターが上昇していること，好塩基球や好酸球からLTC$_4$が産生されやすいこと，アレルゲンチャレンジ後の皮膚において5-LOやcys LTが亢進していること，AD患者の尿中のLTE$_4$値が血清中IgE量と正相関を示すことなどが報告されている．さらにAD患者において，CRTH2陽性T細胞数が増加していることも知られる[14]．

※3 アトピー性皮膚炎（atopic dermatitis：AD）
アトピー性皮膚炎は，痒みを伴う慢性湿疹であり，皮膚のバリア機能異常やIgEを介したTh2型アレルギー反応が関与する．

ADと発症機序を共有するマウスアレルギー喘息モデルにおいて，EP3欠損マウスにおける肥満細胞の機能亢進に伴うアレルギー喘息症状の増悪，IP欠損マウスにおけるIgEの亢進[15]，DP欠損マウスにおける喘息症状の軽減[16]が認められる．また，CRTH2遺伝子欠損マウスでは，ADモデルにおいて炎症細胞の浸潤，好酸球の遊走因子であるRANTES，血清中IgE値の減弱が認められた[17]．さらにTP欠損マウスは，ハプテン反復塗布誘発ADモデルにおけるIgE亢進とともに，強い即時型反応が誘発され，TXA$_2$によるAD発症の抑制作用を示唆するが，その役割は一定の見解が得られていない[8]．したがって，アレルギー炎症において，EP3，IP，TPやPGE$_2$が抑制性であり，DPやCRTH2が亢進性のメディエーターである可能性が指摘されている[2]．

また，LTB$_4$やLTC$_4$は好酸球の化学遊走作用を有しており，ADの遅発相の形成に重要な役割を果たすことが示唆され，抗LT薬が好酸球顆粒タンパク質であるECP値を減少させることも報告された．以上よりアレルギー炎症において，EP3やIP，TPが抑制性，DP，CRTH2，cys LT，LTB$_4$が亢進性のメディエーターや受容体として作用することが推測される．

以上のようにこの数年で小動物を用いたADにおける脂質メディエーターの役割に関する報告が相次いだ．これまでのところ脂質メディエーター関連の薬物がADにおいて有効とする確実な報告はないが，今後，ADの病態形成における脂質メディエーターの役割のさらなる解明と，DP，CRTH2，cys LT1，cys LT2やBLTのアンタゴニストやEP3，IP，TPのアゴニストなどの臨床応用への可能性に期待したい．

4 蕁麻疹と脂質メディエーター

蕁麻疹は喘息と同じく肥満細胞を中心とするI型のアレルギー機序によるところが大きく，これまでは肥満細胞が産生するヒスタミンを阻害する抗ヒスタミン薬が治療の中心を占めてきた．一方で，活性化肥満細胞が産生するcys LTを局注すると，持続性の紅斑・血管拡張が誘発され，この作用は抗ロイコトリエン薬で阻害できる．また，cys LTはcys LT1受容体を介して，好酸球からのIL-4産生や肥満細胞からのIL-5，TNF-α，MIP-1β産生を促し，さらに肥満細胞のcys LT2

受容体を介してIL-8産生を行う．またLTC_4合成酵素あるいはcys LT1受容体欠損マウスにおいて受身皮膚アナフィラキシー反応が約50％抑制されることより，LTC4-cys LT1シグナルの関与が推測される．実際，慢性蕁麻疹にLO阻害薬やロイコトリエン拮抗剤が有効であったという報告や抗ヒスタミン剤に抗LT剤を追加することで有効性が向上したという報告が散見されるが，反応性に個人差が認められるようである．

5 その他の皮膚疾患と脂質メディエーター

元来循環系において研究が進められてきたS1Pが，近年免疫において重要な役割を果たしていることが明らかになった．S1P受容体の1つであるS1P1をリンパ球で欠損させたマウスではT細胞が胸腺や二次リンパ組織から末梢血中に移動することができない．新たな免疫抑制剤として注目されているFTY720は，血中でリン酸化されS1Pと類似した構造になり，リンパ球上のS1P1に作用して受容体の発現レベルを低下させ，末梢リンパ球減少を引き起こしその作用を発揮する．FTY720には植皮の生着の延長効果が報告されている．炎症細胞の局在メカニズムは従来ケモカインや接着因子を中心に進められてきたが，LTをはじめとする脂質メディエーターにも同様の役割があることは興味深い．

その他の皮膚疾患として，尋常性乾癬では以前より好中球の皮膚への浸潤にLTB_4が重要であるとされたが，現在のところLT拮抗薬の有効性は明らかにされていない．また，近年，国内未承認ではあるが，5-LO阻害薬であるzileutonが皮脂の分泌を抑え，尋常性ざ瘡の患者に有効であったという報告がある．このように脂質メディエーターはさまざまな皮膚免疫・アレルギー疾患で役割を果たしていることが推測される．

おわりに

本稿では，遺伝子改変マウス技術を中心としてもたらされた脂質メディエーターの皮膚免疫における新たな役割を述べてきた．注目していただきたいのは，脂質メディエーターがcontext-dependentに多彩な役割を果たすことであり，また，上記の研究成果の多くは，日本人が中心となって世界をリードしてきたということである．今後は，小動物実験にとどまらずヒトでの機能解析や，各種生体刺激後の各脂質メディエーターの産生とその受容体発現変化を網羅的に解析していくことが求められる．脂質メディエーターは局所で作用するオータコイドの一種であるため，受容体レベルでのその作用の制御は副作用の少ない薬剤となりうる可能性が高い．今後，特異的なアゴニストやアンタゴニストを用いて抗アレルギー・免疫疾患薬の開発が進むことが期待される．

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Prostanoid Receptors as Possible Targets for Anti-Allergic Drugs: Recent Advances in Prostanoids on Allergy and Immunology

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Abstract: Prostanoids, consisting of prostaglandins and thromboxane, are cyclooxygenase metabolites of arachidonic acid released in various pathophysiological conditions which exert a range of actions mediated through their respective receptors expressed on target cells. Although it has been difficult to analyze the physiological role of prostanoids, recent developments in both the disruption of the respective gene and receptor selective compounds have enabled us to investigate the physiological roles for each receptor. It has been demonstrated that each prostanoid receptor has multiple functions, and that their expression is regulated in a context-dependent manner that sometimes results in opposite, excitatory and inhibitory, outcomes. The balance of prostanoid production and receptor expression has been revealed to be important for homeostasis of the human body. Here, we review new findings on the roles of prostanoids in allergic and immune diseases, focusing on contact dermatitis, atopic dermatitis, asthma, rheumatoid arthritis, and encephalomyelitis, and also discuss the clinical potentials of receptor-selective drugs.

Keywords: Prostanoid, atopic dermatitis, contact dermatitis, NSAID, prostaglandin, asthma, rheumatoid arthritis, encephalomyelitis, allergy.

INTRODUCTION

Allergic and immune diseases, including asthma, atopic dermatitis (AD), rhinitis, and autoimmune diseases are related to each other as steps in the 'atopic march,' and are found to be increasing in number [1]. They have been treated by suppressing inflammation mainly through steroid-based therapy that, unfortunately, has multiple side effects, such as obesity, hyperglycemia, and osteoporosis, among others. Recently, several advanced therapies for these allergic and immune diseases [2, 3] have been developed, but most of them are very expensive. Therefore, the development of more effective and inexpensive treatments with fewer side effects is in high demand.

When tissues are exposed to diverse pathophysiological stimuli, arachidonic acid (AA) is released from membrane phospholipids, and converted to lipid mediators, such as prostanoids, leukotrienes (LTs) and hydroxy-eicosatetraenoic acids (HETEs). Prostanoids are formed by the cyclooxygenase (COX) pathway, whereas LTs and HETEs are formed by the 5-, 12- and 15-lipoxygenase (LO) pathways. COX has two isoforms, COX-1 and COX-2. While COX-1 is constitutively expressed in cells, COX-2 requires specific stimulation, by substances such as acetone and the phorbol ester TPA [4]. This reaction results in the formation of an unstable endoperoxide intermediate prostaglandin (PG) H_2, which, in turn, is metabolized to PGD_2, PGE_2, $PGF_{2\alpha}$, PGI_2, and thromboxane (TX) A_2 by specific synthases.

Prostanoids are released from cells immediately after formation. Because they are chemically and metabolically unstable, they usually function only locally through membrane receptors on target cells [5]. Nine types and subtypes of membrane prostanoid receptors are conserved in mammals from mouse to human: two subtypes of the PGD receptor (DP; and the chemoattractant receptor homologous-molecule expressed on Th2 cells, CRTH2), four subtypes of the PGE receptor (EP1, EP2, EP3, and EP4), the PGF receptor (FP), the PGI receptor (IP), and the TXA receptor (TP) (Fig. 1). All are G protein–coupled rhodopsin-type receptors with seven transmembrane domains (Fig. 1). Main signal transduction mechanisms of these prostanoid receptors are through regulation of intracellular cyclic adenosine monophosphate (cAMP) concentration and intracellular free calcium concentration. DP, EP2, EP4 and IP are Gs coupled receptors and elevate intracellular cAMP concentration, while EP3 and CRTH2 are Gi coupled receptors and decrease intracellular cAMP. EP1, FP and TP are Gq and other G protein coupled receptors and increase intracellular calcium concentration [5]. However, most of them may couple to more than one G protein with each signaling pathway. Recently, individual prostanoid receptor gene-deficient mice have been used as models to dissect out the respective roles of each receptor in combination with the use of compounds that selectively bind to prostanoid receptors as agonists or antagonists [6]. These genetic and pharmacological approaches have revealed new roles for prostanoids and their receptors in allergic and immune diseases. In this review, we describe the current investigative status of prostanoids in allergic and immune diseases, especially focusing on skin disease, and discuss the clinical potentials of receptor-selective drugs.

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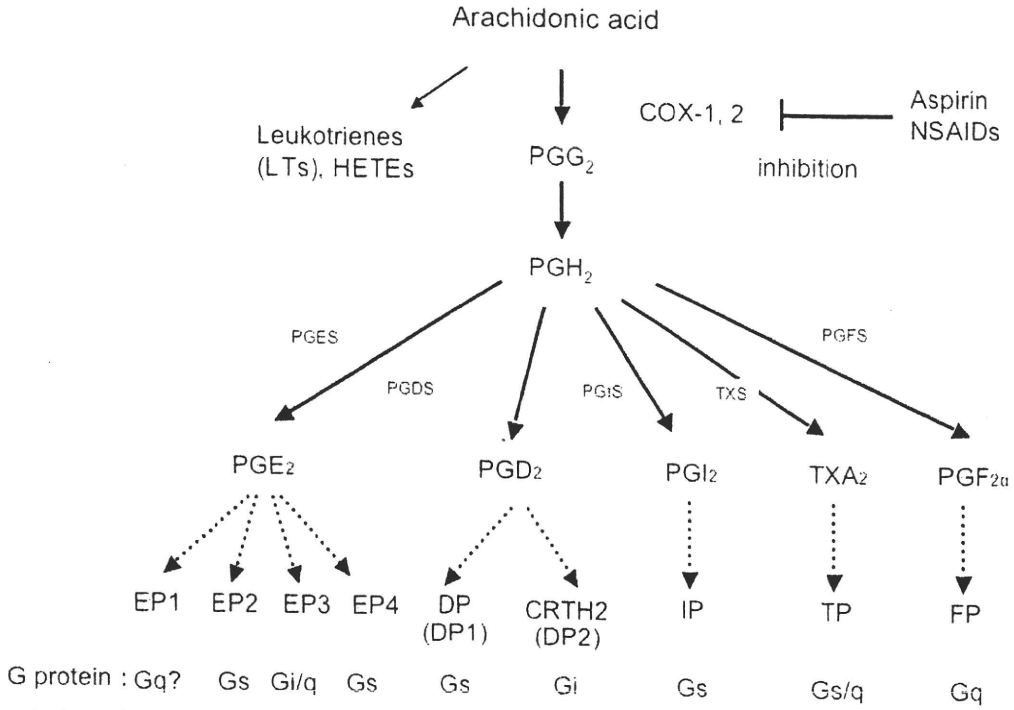

Fig. (1). Biosynthetic pathways of prostanoids. The formation of PGD_2, PGE_2, $PGF_{2\alpha}$, PGG_2, PGH_2, and PGI_2, and TXA_2, from arachidonic acid is shown. The first two steps of the pathway (*i.e.*, conversion of arachidonic acid to PGG_2 and then to PGH_2) are catalyzed by cyclooxygenase (COX), and the subsequent conversion of PGH_2 to each PG is catalyzed by the respective synthase as shown. All are G protein-coupled rhodopsin-type receptors.

PROSTANOID FORMATION IN THE SKIN

Human bodies are exposed to external stimuli continuously. As a representative organ, the skin plays an important role in self-defense during exposure to foreign antigens, and consists of many immune cells, such as keratinocytes (KCs), T cells, B cells, mast cells, eosinophils, fibroblasts, and two types of cutaneous dendritic cells (DCs), epidermal Langerhans cells (LCs) and dermal DCs (dDCs). In the normal human skin, immunohistochemical examinations have revealed that COX-1 is observed throughout the epidermis, whereas COX-2 exists in more differentiated, suprabasilar KCs and outer root sheath cells of hair follicles [7, 8]. Among prostanoids, PGE_2 is the main COX product in human epidermal homogenates [9]. PGD_2 has been detected in human skin [9], and PGD synthase is present predominantly in LCs, dDCs, dermal macrophages and mast cells, but not in KCs [10, 11]. Of this group of cells, mast cells have been found to be one of the major cellular sources of PGD_2. Only very low TX synthase activity has been found in the skin; however, high levels of TXB_2, as a metabolite of TXA_2, were detected in the cultured supernatant of LCs and DCs [12]. PGI_2 was detected in the skin of the murine atopic dermatitis model [13]. $PGF_{2\alpha}$, was observed in skin exudates of nickel allergy patients [14]. The above findings on the synthesis of prostanoids are summarized in Table 1.

Table 1. Expression of Prostanoid Synthases and Prostanoid Receptors in the Skin

	PGDS	PGES	PGFS	PGIS	TXS	DP	CRTH2	EP1	EP2	EP3	EP4	FP	IP	TP
Keratinocytes	m	m, h						h	h	h	h			
Langerhans cells	h	m, h			m	m, h		m	m	m	m			
Dendritic cells	h	m, h		m	m, h				m, h		m, h		m	
T cells							m, h(Th2)	m	m	m	m, h		m	m
B cells								m, h	m, h	m, h	m, h		m	
Macrophages	h	m, h		m	m, h		m	m	m	m	m, h			
Eosinophils	h	h	h		h	m	m		m, h		h			h
Mast cells, basophils	m, h				h	m, h	m, h	m	m	m	m			
Neutrophils		h			h	h	m		m, h		m			
Blood vessels		h	h	h				m, h	m, h	m, h	m, h		m, h	m, h

PG; Prostaglandin, s; synthase, m; mouse, h; human
Modified from the reference by Tilley *et al.*

PROSTANOID RECEPTOR EXPRESSION ON KERATINOCYTES AND IMMUNE CELLS

Adult human KCs express mRNA for all subtypes of PGE_2 receptors [15, 16] and the expression of all PGE_2 receptors have been detected in mouse KCs by immunohistochemistry [17]. Mouse LCs and DCs have been shown to express DP [18], EP1, 2, 3, 4 [19], and IP [20]. T cells are known to express EP1, 2, 3, 4 [21], IP [22] and TP [12]. PGE_2 suppresses T cell proliferation, T cell differentiation in the thymus, and IL-1 production by acting at EP2 and EP4 [23] *in vitro*. B cells express EP1, 2, 3, and 4 [21] and PGE_2 facilitates IgE class switching through EP2 and EP4 *in vitro* [24]. Mast cells express EP1, 2, 3, 4, DP, and IP [21, 25], and PGE_2 acts at EP3 to suppress degranulation [26]. Human eosinophils express EP2, EP4, DP, CRTH2 and TP [25, 27], and PGE_2 seems to prolong eosinophil survival [28, 29]. On the other hand, PGE_2 suppresses TNF-α production and enhances IL-6 production from neutrophils stimulated by lipopolysaccharide (LPS) through EP2 and EP4 [25, 30]. As summarized in Table **1**, prostanoids and their receptors are produced and expressed by a wide variety of cells in the skin. This varied expression pattern of prostanoids maintains the homeostasis of our body, which will be discussed as below.

NON-STEROIDAL ANTI-INFLAMMATORY DRUGS AND PROSTANOIDS

The roles of prostanoids on allergy and immune diseases have been suggested by clinically monitoring the effects of non-steroidal anti-inflammatory drugs (NSAIDs), a set of COX inhibitors. It is well known that cutaneous immune responses are associated with an increase in prostanoid formation; however, the roles of prostanoids have been less well defined. This is presumably because the effects of NSAIDs are far less marked compared to those of steroids [31]. There are some skin diseases that are effectively treated with NSAIDs [3, 31, 32]; for example, ibuprofen piconol has an anti-inflammatory effect on acne vulgaris, by inhibiting leukocyte migration induced by *Propionibacterium acnes* [33]. However, NSAIDs are generally not useful for inflammatory skin diseases, such as contact dermatitis and AD [3, 31] largely because NSAIDs occasionally induce contact dermatitis themselves. Since NSAID is a COX inhibitor, which blocks formation of all prostanoids, observations obtained from NSAIDs neither indicate which type of prostanoid nor which class of prostanoid receptor is involved in a given process. Recent genetic and pharmacological approaches have revealed some unexpected findings regarding each prostanoid receptor. It is high time to reconsider the significance of each prostanoid receptor in allergic and immune diseases.

PROSTANOIDS IN CONTACT HYPERSENSITIVITY - SENSITIZATION PHASE

In order to evaluate the physiological roles of prostanoids in immune responses of the skin, the use of a contact hypersensitivity (CHS) model (in other words, allergic contact dermatitis) is an effective tool [34, 35]. Migration of cutaneous DCs to the lymph nodes is a crucial step in the initiation of CHS. This activation of cutaneous DCs is initiated by KCs that secrete pro-inflammatory cytokines upon antigen application. Thus, by virtue of their specific cytokine secretion pattern, KCs determine the microenvironment for cutaneous DC maturation and migration (Fig. **2**).

PGE_2 produced by KCs upon antigen exposure acts at EP4 on cutaneous DCs to facilitate initiation of cutaneous immune responses by promoting the migration and maturation of cutaneous DCs, and the blockade of PGE_2-EP4 signaling attenuates the CHS response [19] (Fig. **2**). Interestingly, prostanoid activity producing the opposite effects has also been documented: PGD_2 induced by percutaneous infection with the helminth parasite *Schistosoma mansoni* specifically impedes the migration of LCs through the DP receptor [18], and administration of the DP agonist, BW245C, inhibits migration of LCs and attenuates OVA-induced dermatitis [36]. Stimulation of DP signaling also inhibits the migration of lung DC, which leads to the suppression of airway inflammation [37]. The PGI_2 receptor IP inhibits the proinflammatory cytokine production and T cell stimulatory function of DCs [38]. These activities of lipid mediators are not only limited to prostanoids: LC migration from the skin to the lymph nodes utilizes the LTC_4 transporter multidrug resistance-associated protein 1 [39]. The significance of these prostanoid receptors in pathophysiological conditions remained to be elucidated, but modulation of the signaling of these receptors may lead to a discovery of a possible candidate for the immune reaction.

Once cutaneous DCs migrate to draining lymph nodes, they present antigens to naïve T cells to prime them. Subsequently, the engagement of the antigen complex by T cell receptors triggers clonal expansion and differentiation of T cells. $CD4^+$ helper T (Th) cells are differentiated into at least three subsets: Th1, Th2 and Th17. Similarly, $CD8^+$ cytotoxic T (Tc) cells undergo differentiation into two subsets: Tc1 cells and Tc2 cells. Contact hypersensitivity is mainly mediated by Th1 cells and to some extent by Th17 cells [40]. Although the suppressive activity of PGE_2 on Th1 differentiation *in vitro* has been known since the 1980s, the *in vivo* role of PGE_2 on Th differentiation has only recently been addressed. In the sensitization phase of CHS, PGE_2 produced by DCs stimulate EP1 receptors on naïve $CD4^+$ and $CD8^+$ T cells and promote Th1 and Tc1 differentiation [41]. Accordingly, EP1-deficient mice showed reduced Th1 and Tc1 differentiation and CHS responses [41]. In addition to EP receptor signaling, IP signaling promotes Th1 and Tc1 differentiation through cAMP dependent mechanism [42]. Interestingly, it has been reported that IP deficient mice showed enhanced Th2 response such as elevated IgE concentration in serum in mouse OVA-induced asthma model [22], suggesting that lack of PGI_2-IP signaling might result in Th2 biased immune response through inhibition of Th1 differentiation in IP deficient mice. Prostanoids also regulate DC-T cell interaction in the priming of naïve T cells. Cutaneous DCs produce abundant TXA_2, which acts on naïve T cells to impair the DC-T cell interaction [12]. Predictably, TP-deficient mice or wild-type mice treated with a TP antagonist, S-145, during the sensitization period showed enhanced CHS responses, indicating that TP signaling negatively regulates the priming of T cells [12] (Fig. **2**).

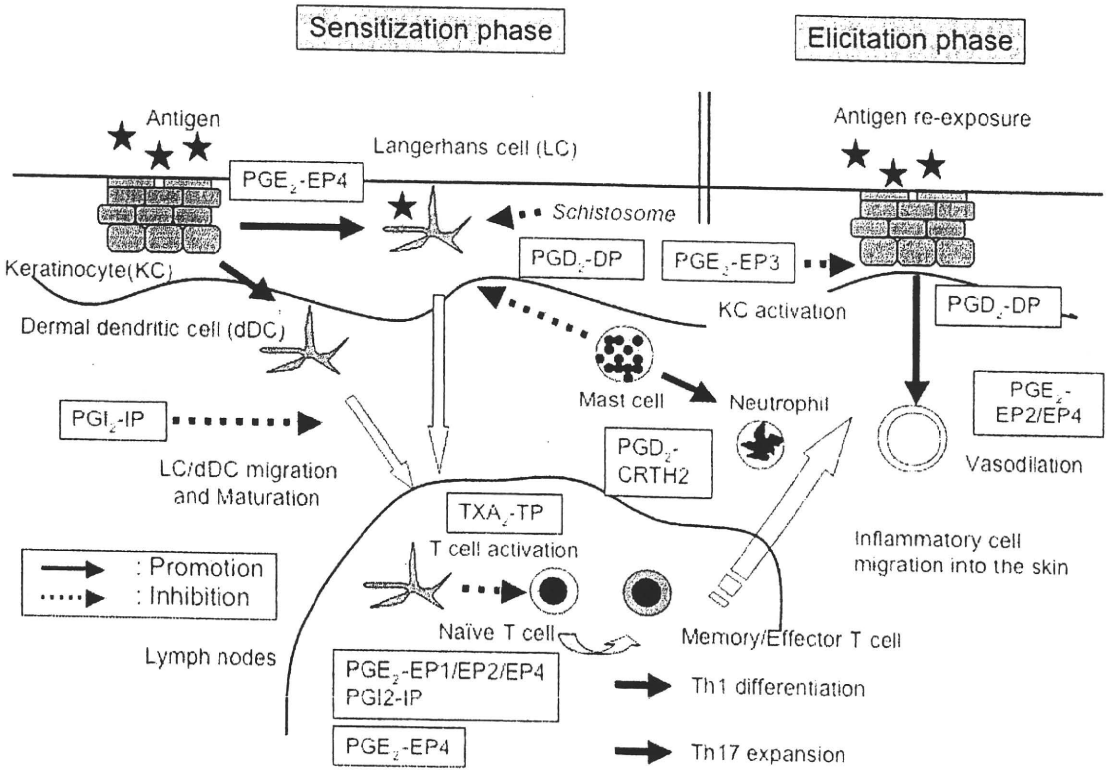

Fig. (2). Hypothesis on the role of prostanoids in the contact hypersensitivity response. During the sensitization period, antigen induces pro-inflammatory cytokine secretion by KCs, which enhances cutaneous DCs (LCs and dDCs) activation and migration to regional lymph nodes. In the lymph nodes, cutaneous DCs activate naïve T cells which differentiate into mature memory T cells. During antigen exposure to the skin, KCs produce PGE_2, and mast cells produce PGD_2. Moreover, *schistosomes* produce PGD_2 during helminthic infection. The PGE_2-EP4 pathway promotes, but PGD_2-DP and PGI_2-IP pathways inhibit cutaneous DC migration and maturation. TXA_2 produced by activated cutaneous DCs seems to act on naïve T cells to disrupt DC-T cell interaction. The PGE_2-EP1/EP2/EP4 pathways promote Th1 cell differentiation. The PGE_2-EP4 pathway also promotes Th17 cell expansion.

During the elicitation phase of the CHS response, secondary antigen exposure to the skin stimulates KCs to secrete pro-inflammatory cytokines, chemokines and other mediators, which activate the endothelial activation of blood vessels. This activation attracts memory T cell infiltration into the skin. Subsequently, antigen-loaded antigen-presenting cells activate memory T cells to induce mediator release. PGE_2 dilates blood vessels possibly through EP2 and EP4. PGE_2-EP3 signaling inhibits KCs activation and plays an anti-inflammatory role in CHS.

Regulatory T cells (Tregs) are another important T cell subset, which suppress the activation of effector T cells and play suppressive function in various disease, including CHS [43-45] It has been reported that PGE_2 promote Foxp3 mRNA expression throught EP2 and EP4 dependent mechanism *in vitro* [46]. However, the physiological role of these signaling in the skin immune response has yet to be elucidated.

6. PROSTANOIDS IN CONTACT HYPERSENSITIVITY – ELICITATION PHASE

After establishment of the sensitization phase, antigen re-challenge onto the skin stimulates KCs to produce memory T cell-attracting chemokines, such as CCL27, and neutrophil-attracting chemokines, such as CXCL1 and CXCL2, and to evoke inflammation, in a stage called the elicitation phase. It has been demonstrated that these chemokines are induced by PGE_2 [35, 47], and several prostanoid receptors are also involved in this phase. For example, PGD_2 promotes neutrophil infiltration through CRTH2 and contributes to the progression of inflammation [48]. Accordingly, administration of CRTH2 antagonist attenuates the CHS response [49]. On the other hand, stimulation of the EP3 receptors on KCs inhibited the chemokine expression in KCs, and suppressed the CHS response [50]. Predictably, EP3-deficient mice showed increased CHS responses, suggesting that both endogenous and exogenous EP3 signaling plays an anti-inflammatory role at the elicitation site under certain conditions [50].

On the other hand, TPA is known to induce skin inflammation. After TPA application, acute edema is induced and followed by inflammatory cell infiltration, with this entire episode being induced by TNF–α and PGE_2 [51]. In fact, intradermal injection of PGE_2 into human skin causes erythema with vascular permeability changes [52-54]. Similar skin inflammation is induced by ultraviolet B, which is mediated through EP2 and EP4 receptors [55]. Such direct effects of prostanoids on blood vessels might affect the elicitation

phase of CHS, but the physiological role in this context remains unknown.

7. PROSTANOIDS IN AD

AD is a common pruritic and chronic inflammatory skin disease that is regarded as one of the T helper type 2 (Th2) diseases. In the dermis, a cellular infiltrate is present consisting of lymphocytes, monocytes and mast cells. Histamine is one of the mediators suspected to play an important role in Th2 disease processes, but its role in AD is uncertain because antihistamines improve the disease only partially, not dramatically [56, 57]. Therefore, in the search for potential mediators involved in the inflammatory processes of AD, mediators other than histamine have to be considered. In this respect, it would be of relevance to examine the potential role of prostanoids in the molecular pathology of AD. In biopsy specimens from patients with AD, PGE_2 has been determined in biologically active amounts in both lesional and perilesional skin [58]. In contrast, normal levels of eicosanoids were found in the uninvolved skin of these patients [58]. Using an ovalbumin-induced mouse AD model, COX-2 inhibition induced both enhanced eosinophil infiltration and elevated IL-4 expression in the skin lesion with elevated serum IgE and IgG1, suggesting that COX-2-derived prostanoids play protective roles in the development of AD. As TP-deficient mice exhibited an enhanced immune response with an increased serum IgE level on a repeated hapten application-induced murine AD model [12], TP signaling may be responsible for the worsened phenotype of COX-2-deficient mice.

PGE_2 has the capacities to induce wheal and flare reactions when injected into human skin [59] and to modulate the inflammatory responses elicited by other mediators [54, 60]. In contrast, one of the characteristics of AD is the elevation of IgE in the sera of patients, which is related to pruritus [61]. PGE_2 drives Ig class switching to IgE by acting at EP2 and EP4 on B cells under LPS and IL-4 stimulation *in vitro* [24]. The physiological role of PGE_2 on class switching in AD patients should be pursued in future studies.

PGD_2 is the major prostanoid produced by activated mast cells. PGD_2 has two types of receptors, DP and CRTH2. CRTH2 induces chemotaxis in Th2 cells, eosinophils and basophils with enhanced degranulation [62, 63]. In response to PGD_2, CRTH2 also induces Th2 cell and neutrophil migration into inflammatory skin sites [48]. Virtually all $CRTH2^+$ $CD4^+$ lymphocytes have a pure Th2 phenotype and occupy not all but a large proportion of circulating Th2 cells in both normal and AD subjects. In AD patients, a preferential increase of $CRTH2^+$ cells was noted within the disease-related cutaneous lymphocyte-associated antigen-positive $CD4^+$ T cell compartment [64]. There remains a need to clarify the respective roles of DP and CRTH2 in pathophysiological conditions.

Pruritus is also an important hallmark of AD as PGE_2 is known to evoke pruritus in AD patients [65]. And PGD_2, but not the CRTH2 agonist, 13, 14-dihydro-15-keto-PGD_2, reduced scratching behavior in NC/Nga AD model mice, suggesting that DP suppresses pruritic activity [66].

The above findings indicate that each prostanoid receptor plays its own role in a context-dependent manner (Fig. **3**).

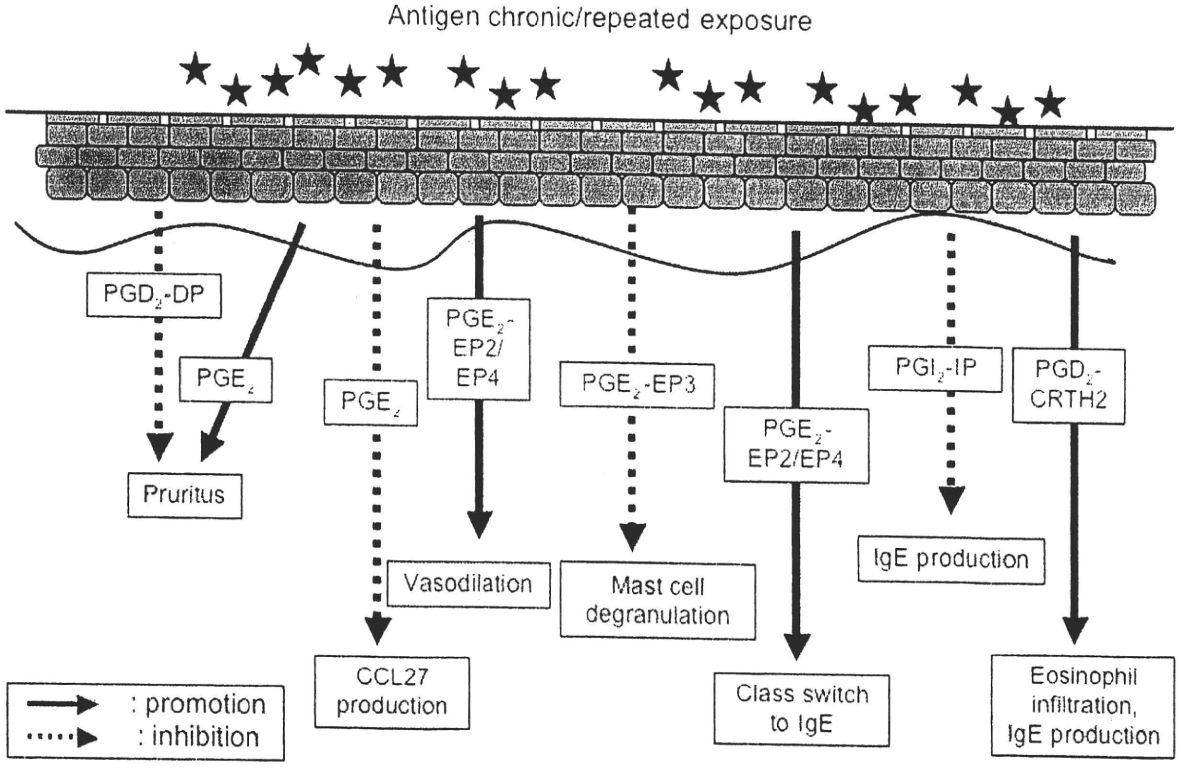

Fig. (3). Prostanoids in atopic dermatitis. A considerable amount of recent data has suggested that prostanoid receptors play some critical roles in the pathogenesis of AD in a context-dependent manner. Possible roles are summarized.

AD might be controlled by antagonists for DP, CRTH2, EP2, EP4, and/or IP, and by agonists for EP4 and/or TP.

8. PROSTANOIDS IN ASTHMA

PGD_2 is a major prostanoid produced by activated mast cells [67] and is released in large amounts during asthmatic attacks in certain patients [68]. It has also been reported that the increase of prostanoid in BALF were detected in a mouse asthma model [69, 70]. Although the role of PGD_2 in allergic asthma long remained unclear, an analysis using DP-deficient mice revealed that PGD_2-DP signaling stimulates chemokine expression on airway epithelial cells and facilitates Th2 cell and eosinophil accumulation in the lungs, and plays a central role in asthma [67]. Single nucleotide polymorphism analysis of the human DP gene (PTGDR) also suggests the importance of DP in the pathogenesis of asthma [71]. From these observations, it suggests that prostanoids facilitate asthmatic attacks through DP signaling both in mice and humans.

In addition to DP actions, an involvement of PGD_2-CRTH2 signaling has been reported in asthma. Administration of a CRTH2 antagonist reduced eosinophil accumulation in a mouse asthma model, and administration of a CRTH2 agonist augmented infiltrations of inflammatory cells into the lungs [72, 73], suggesting that PGD_2-CRTH2 signaling also mediate airway inflammation. However, CRTH2-deficient mice showed increased inflammatory cell infiltrations and IL-5 production from activated T lymphocytes [74], suggesting that CRTH2 signaling may regulate cytokine production in the development of asthma. Further analyses are needed to clarify whether an inhibition of CRTH2 signaling would have an overall beneficial effect.

On the other hand, the existence of prostanoid receptors that negatively regulate allergic reactions has been reported [26]. Among PGE receptor-deficient mice, EP3-deficient mice showed exaggerated airway inflammation, and administration of an EP3 agonist suppressed the inflammation by inhibiting mast cell activation and chemokine production from airway epithelial cells [26]. EP3 signaling is also reported to play an anti-inflammatory role in experimental allergic conjunctivitis [75]. These results indicate that PGE_2-EP3 signaling negatively regulates allergic inflammation. It has been well known that ingestion of aspirin (NSAIDs), which blocks prostanoid synthesis, sometimes induces severe bronchoconstriction in a proportion of subjects with asthma. Previously, such aspirin-induced asthmatic attacks (AIA) were explained by the diversion of arachidonic acid metabolism from the COX pathway to the LO pathway. However, the balance of PGD_2-DP signaling as promoter of airway inflammation and PGE_2-EP3 signaling as suppressor of airway inflammation may explain the mechanism of the AIA.

9. PROSTANOIDS IN RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is a chronic inflammatory disease of the joints characterized by inflammatory cell

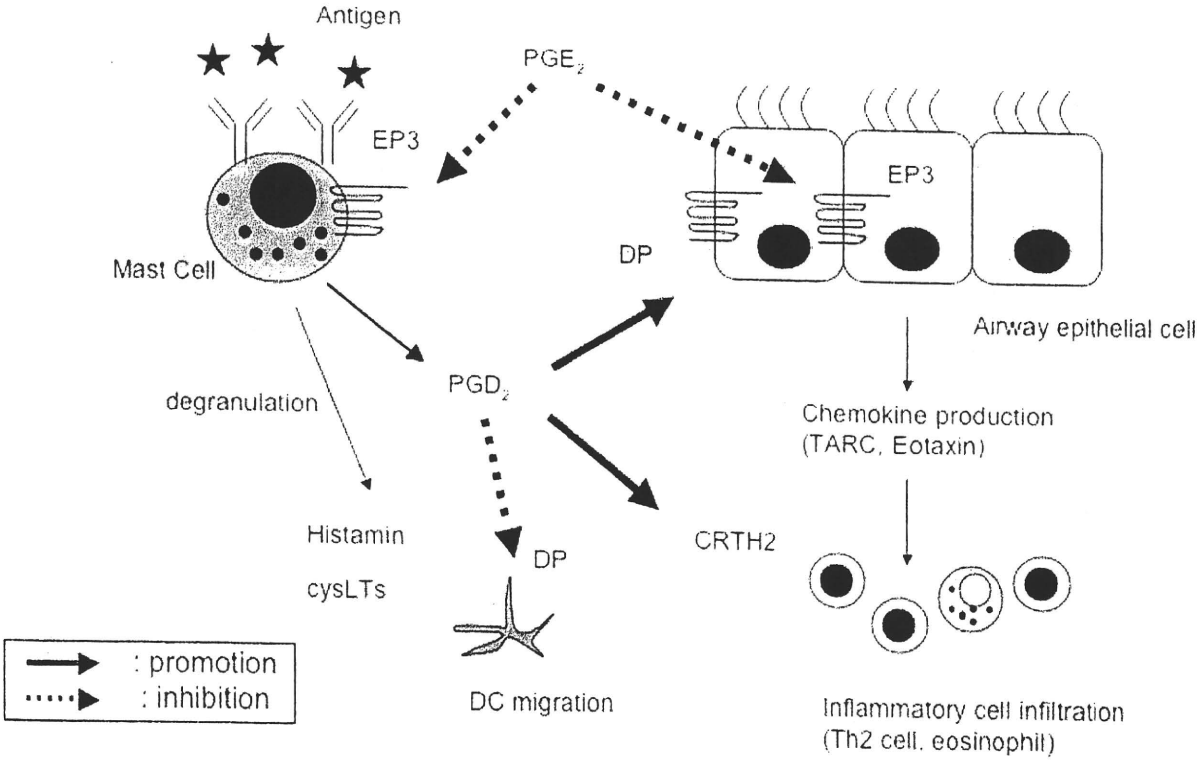

Fig. (4). Prostanoids in asthma. PGD_2 produced by mast cells act on airway epithelial cells through DP and facilitate inflammation by promoting chemokine production. PGD_2-DP signaling plays a suppressive role in the sensitization phase by inhibiting DC migration and activation. PGD_2 also acts on Th2 cells and eosinophils through CRTH2, and promotes accumulation of inflammatory cells. In contrast, PGE_2-EP3 signaling plays an anti-inflammatory role by inhibiting mast cell degranulation and chemokine production from airway epithelial cells.

infiltration, synovial hyperplasia and destruction of cartilage and bone. NSAIDs have been long and widely used for treatment of RA. Among PGs, PGE_2 has been suggested as a main PG type active in RA reactions. In fact, it has been reported that mice deficient in microsomal PGE synthase-1 showed reduced arthritic responses in mouse collagen-induced arthritis (CIA) [76]. It was later revealed that EP2 and EP4 mediate inflammation in CIA [77]. Furthermore, PGI_2-IP signaling plays a critical role in the development of CIA by enhancing the expression of arthritis related genes, such as IL-6 vascular endothelial growth factor-A, and the receptor activator of NF-kappa B ligand, in synovial fibroblasts [78]. Regulation of both PGI_2-IP signaling and PGE_2-EP2/EP4 signaling can be one of the potential targets in controlling joint inflammation.

10. PROSTANOIDS IN ENCEPHALOMYELITIS

Recently, EP2 and EP4 receptors have been reported to regulate Th1 and Th17 differentiation [79]. Both EP2 and EP4 signaling on naïve T cells promote Th1 differentiation through the phosphatidyl inositol-3 kinase pathway. *EP4 signaling also promote Th17 differentiation through the cAMP pathway, and blockade of EP4 signaling inhibited Th17 differentiation in CHS and mouse experimental encephalomyelitis (EAE) [79].* As Th17 cells are involved in many diseases, including not only CHS, but also psoriasis, rheumatoid arthritis, and encephalomyelitis among others, an antagonist of the EP4 receptor may become a useful drug target for regulating Th17-mediated diseases.

11. CONCLUSIONS

In this review, we have summarized current findings on the actions of prostanoids and their receptors in allergic and immune diseases of the skin. It is worthwhile to mention the role of prostanoid receptors on cutaneous DC function, where EP4 as promotor of DC migration and DP as inhibitor of DC migration mediate in opposite directions. NSAID treatment may therefore mask the complex effects of prostanoids in the disease pathway. Clarification of each prostanoid pathway should widen our understanding not only of the actions of prostanoids but also of the delicate regulation of cutaneous immune reactions. At present, the studies on the role of allergic and immune diseases at the prostanoid receptor level were mostly conducted using experimental animals. The next question to address is to what degree this pathway contributes to initiation and progression of human diseases, and how effective the therapy directed to this signaling is. There are several ongoing efforts to develop better prostanoid receptor agonists and antagonists, and clinical trials involving these types of drugs may be able to clarify these issues. Selective manipulation of the actions mediated by each receptor may provide a novel therapeutic strategy for cutaneous allergic or inflammatory disorders.

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Molecular Pathogenesis of Genetic and Inherited Diseases

Flaky Tail Mouse Denotes Human Atopic Dermatitis in the Steady State and by Topical Application with *Dermatophagoides pteronyssinus* Extract

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The barrier abnormality, a loss-of-function mutation in the gene encoding filaggrin (*FLG*), which is linked to the incidence of atopic dermatitis (AD), is a recently discovered but important factor in the pathogenesis of AD. Flaky tail (*Flg^{ft}*) mice, essentially deficient in filaggrin, have been used to investigate the role of filaggrin on AD. However, the relevancy of *Flg^{ft}* mice to human AD needs to be determined further. In this study, we observed the clinical manifestations of *Flg^{ft}* mice in the steady state and their cutaneous immune responses against external stimuli, favoring human AD. Under specific pathogen-free conditions, the majority of *Flg^{ft}* mice developed clinical and histological eczematous skin lesions similar to human AD with outside-to-inside skin barrier dysfunction evaluated by newly devised methods. In addition, cutaneous hapten-induced contact hypersensitivity as a model of acquired immune response and a mite extract-induced dermatitis model physiologically relevant to a human AD were enhanced in *Flg^{ft}* mice. These results suggest that the *Flg^{ft}* mouse genotype has potential as an animal model of AD corresponding with filaggrin mutation in human AD. ***(Am J Pathol 2010, 176:2385–2393; DOI: 10.2353/ajpath.2010.090957)***

Atopic dermatitis (AD), which affects at least 15% of children in developed countries, is characterized by eczematous skin lesions, dry skin, and pruritus.[1–3] Although the precise pathogenic mechanism of AD is as yet unknown, several accumulated lines of evidence suggest that a defective skin barrier to environmental stimuli may contribute to its pathogenesis. It has long been thought that the barrier abnormality in AD is not merely an epiphenomenon but rather is the "driver" of disease activity.[4] The evidence for a primary structural abnormality of the stratum corneum in AD is derived from a recently discovered link between the incidence of AD and loss-of-function mutations in the gene encoding filaggrin (*FLG*). Individuals carrying the *FLG* null allele variants tend to develop AD.[5–7]

Filaggrin protein is localized in the granular layers of the epidermis. Profilaggrin, a 400-kDa polyprotein, is the main component of keratohyalin granules.[8–10] In the differentiation of keratinocytes, profilaggrin is dephosphorylated and cleaved into 10 to 12 essentially identical 27-kDa filaggrin molecules, which aggregate in the keratin cytoskeleton system to form a dense protein-lipid matrix.[10] This structure is thought to prevent epidermal water loss and impede the entry of external stimuli, such as allergens, toxic chemicals, and infectious organisms. Therefore, filaggrin is a key protein in the terminal differentiation of the epidermis and in skin barrier function.[11]

Because AD is a common disease for which satisfactory therapies have not yet been established, understanding the mechanism of AD through animal models is an essential issue.[1,12] Flaky tail (*Flg^{ft}*) mice, first introduced in 1958, are spontaneously mutated mice with

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abnormally small ears, tail constriction, and a flaky appearance of the tail skin, which is most evident between 5 and 14 days of age.[13] Mice of the *Flg*^{ft} genotype express an abnormal profilaggrin polypeptide that does not form normal keratohyalin F granules and is not proteolytically processed to filaggrin. Therefore, filaggrin is absent from the cornified layers in the epidermis of the *Flg*^{ft} mouse.[14–16]

Recently, it has been revealed that the gene responsible for the characteristic phenotype of *Flg*^{ft} mice is a nonsense mutation of 1-bp deletion analogous to a common human *FLG* mutation.[15] These mice developed eczematous skin lesions after age 28 weeks under specific pathogen-free (SPF) conditions[17] and enhanced penetration of tracer perfusion determined by ultrastructural visualization,[16] and were predisposed to develop an allergen-specific immune response after epicutaneous sensitization with the foreign allergen ovalbumin (OVA).[15,17] On the other hand, general immunity through intraperitoneal sensitization with OVA was comparable between *Flg*^{ft} mice and control mice.[15,17]

Despite these recent advances, there still remain several issues with *Flg*^{ft} mice to be addressed. For example, serial close observation of clinical manifestations in reference to human AD will be informative. It is of value to evaluate the responses to external stimuli relevant to human AD, such as mite extracts, instead of OVA that has been used previously. A comparative study on the skin-mediated contact hypersensitivity (CHS) response and non-skin-mediated delayed-type hypersensitivity response is important to evaluate the impact of barrier dysfunction on immune responses *in vivo*. In addition, although it has now been determined that the barrier dysfunction is a key element in the establishment of AD, there is no established method to evaluate the outside-to-inside barrier function quantitatively.

In this study, we found that *Flg*^{ft} mice showed spontaneous dermatitis with skin lesions mimicking human AD in a steady state under SPF conditions: serial occurrence of manifestations as scaling, erythema, pruritus, and erosion followed by edema in this order. We also successfully evaluated outside-to-inside barrier dysfunction in *Flg*^{ft} mice quantitatively using a newly developed method. In addition, we determined that the Th1/Tc1-mediated immune response was enhanced by immunization through skin but not through non-skin immunization. Last, we induced severe AD-like skin lesions in *Flg*^{ft} mice by application of mites as a physiologically relevant antigen for human AD, which will be an applicable animal model of AD.

Materials and Methods

Mice

C57BL/6NCrSlc (B6) mice were purchased from SLC (Shizuoka, Japan). Flaky tail (STOCK *a/a ma ft/ma ft*/J; *Flg*^{ft} mice) mice have double-homozygous filaggrin (*Flg*) and matted (*ma*) mutations.[13,14] We used B6 mice as a control of *Flg*^{ft} mice because *Flg*^{ft} mice were described to be outcrossed onto B6 mice at The Jackson Laboratory (Bar Harbor, ME)[13,14] (of note, although the strain was crossed with B6, it is not a B6 congenic strain but rather a hybrid stock that is probably semi-inbred). Female mice were used in all experiments unless otherwise stated; they were maintained on a 12-hour light/dark cycle at a temperature of 24°C and at a humidity of 50 + 10% under SPF conditions at Kyoto University Graduate School of Medicine. Routine colony surveillance and diagnostic workup verified that mice were free of Ectromelia virus, lymphocytic choriomeningitis virus, mouse hepatitis virus, Sendai virus, *Mycoplasma pulmonis*, cilia-associated respiratory bacillus, *Citrobacter rodentium* [*Escherichia coli* O115a,c:K(B)], *Clostridium piliforme* (Tyzzer's organism), *Corynebacterium kutscheri*, *Helicobacter hepaticus*, *Pasteurella pneumotropica*, *Salmonella* spp., parasites, intestinal protozoans, *Enterobius*, and ectoparasites. All experimental procedures were approved by the Institutional Animal Care and Use Committee of Kyoto University Graduate School of Medicine.

Clinical Observation and Histology

The clinical severity of skin lesions was scored according to the macroscopic diagnostic criteria that were used for the NC/Nga mouse.[18] In brief, the total clinical score for skin lesions was designated as the sum of individual scores, graded as 0 (none), 1 (mild), 2 (moderate), and 3 (severe), for the symptoms of pruritus, erythema, edema, erosion, and scaling. Pruritus was observed clinically for more than 2 minutes.

For the histological portion of the study, the dorsal skin of mice was stained with H&E. Toluidine blue staining was used to detect mast cells, and the number of mast cells was calculated as the average from five different fields of each sample ×40 magnification).

Flow Cytometric Analysis and Quantitative RT-PCR

Cells from the skin-draining axillary and inguinal lymph nodes (LNs) and from the spleen were analyzed with flow cytometry. Fluorescent-labeled anti-CD4 and anti-CD8 antibodies were obtained from eBioscience (San Diego, CA) and used to stain cells. The total number of cells per organ and the number of cells in each subset were calculated through flow cytometry using the FACSCanto II system (Becton Dickinson, San Diego, CA). Quantitative RT-PCR was performed as described previously, using the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) as a control.[19]

Total and Mite-Specific Serum IgE

Total serum IgE levels were measured with a mouse IgE ELISA Kit (Bethyl Laboratories, Montgomery, TX) according to the manufacturer's protocols. For the measurement of mite-specific IgE levels, the same type of mouse IgE ELISA Kit was used with slightly modifications. Specifi-

cally, plates were coated and incubated with 10 μg/ml *Dermatophagoides pteronyssinus* (Dp) (Biostir, Kobe, Japan) diluted with coating buffer for 60 minutes. After a blocking period of 30 minutes, 100 μl of 5× diluted serum was added into each well and incubated for 2 hours. Anti-mouse IgE-horseradish peroxidase conjugate (1:15,000; 100 μL) was used to conjugate the antigen-antibody complex for 60 minutes at room temperature; from this point on the ELISA Kit was used according to the manufacturer's protocol. Absorbance was measured at 450 nm. The difference between the sample absorbance and the mean of negative control absorbance was taken as the result.

Skin Barrier Function

The dorsal regions of the skin were shaved in all mice before measurement. To evaluate inside-to-outside barrier function, transepidermal water loss (TEWL) was measured with a Tewameter Vapo Scan (Asahi Biomed, Tokyo, Japan) at 24°C and 46% relative humidity.

Outside-to-inside barrier function was assessed by means of fluorescein isothiocyanate isomer I (FITC) (Sigma-Aldrich, St. Louis, MO). The shaved dorsal skin of mice was treated with 100 μl of 1% FITC diluted in acetone and dibutyl phthalate (1:4); 3 hours later, this area was tape-stripped (Scotch tape, 3M, St. Paul, MN) nine times to remove the stratum corneum containing the remnant of FITC. The painted area (1.2 cm × 1.2 cm) was removed, and FITC concentration was measured. Each skin sample was soaked in PBS at 60°C for 10 seconds, after which the dermis and epidermis were separated. The epidermis was soaked in 500 μl of PBS, homogenized, and spun down at 2200 × *g*. The supernatant was collected, and fluorescence was measured at an excitation wavelength of 535 nm and an emission wavelength of 460 nm using an Arvo SX 1420 counter (Wallac, PerkinElmer, Waltham, MA). The fluorescence value was compared with a standard curve using FITC serial dilutions.

For the evaluation of fluorescence intensities of FITC penetrated into the epidermis, a 1 × 1 cm skin sample was taken after tape stripping, and a 10-μm Tissue-Tek (Sakura Finetek, Tokyo, Japan)-embedded section was analyzed using a BZ-9000 Biorevo digital microscope (Keyence, Osaka, Japan) at the same time exposure.

An *in situ* dye permeability assay with toluidine blue was performed using embryos at 18 days (littermates). Unfixed, untreated embryos were dehydrated by a 1-minute incubation in an ascending series of methanol (25, 50, 74, and 100%) and rehydrated with the descending same methanol series, washed in PBS, and stained with 0.01% toluidine blue.

Scratching Behavior

Scratching behavior was measured in detail using the Sclaba Real system (Noveltec, Kobe, Japan). Mice were put into the machine 20 minutes before measurement to allow them to adapt to the new environment. Ointment was then applied, and the number and duration of scratching sessions were counted according to the manufacturer's protocol for 15 minutes.[20]

Dermatitis Models

For the assessment of irritant contact dermatitis, 20 μl of 0.2 mg/ml phorbol myristate acetate (PMA) (Sigma-Aldrich) was applied to both sides of the ears. Ear thickness change was measured at 1, 3, 12, and 24 hours as well as 5 days after application.

To induce a CHS response, 25 μl of 0.5% 1-fluoro-2.4-dinitrobenzene (DNFB) (Nacalai Tesque, Kyoto, Japan) was painted on the shaved abdomens of mice for sensitization. Five days later, the ears were challenged with 20 μl of 0.2% DNFB, and ear thickness change was measured at 24 and 48 hours after application. Nonsensitized mice were used as a control. A delayed-type hypersensitivity response model was established using OVA (Sigma-Aldrich). Mice were sensitized with 200 μl of 0.5 mg/ml of OVA in complete Freund's adjuvant (Difco Laboratories, Detroit, MI) intraperitoneally and challenged 5 days later with an injection of 20 μl of 1 mg/ml of OVA in incomplete Freund's adjuvant (Difco Laboratories) into the hind footpads. Footpad thickness was measured before and 24 hours after challenge. Nonsensitized mice were used as a control. Footpad swelling was calculated by (footpad thickness change of sensitized mice) − (footpad thickness change of nonsensitized mice). To induce murine AD-like skin lesions, 40 mg of 0.5% Dp in white petrolatum was topically applied to the ears and upper back twice a week for 8 weeks. Petrolatum without Dp was used as a control. One gram of Dp body product (Biostir) contained 1.78 mg of total protein with 2.47 μg of Dp protein (Der p1). Ear thickness and clinical scores were measured every week. Mite-specific IgE levels, TEWL, and histological appearance of eczematous skin were observed 12 hours after the final application.

Statistical Analysis

Data were analyzed using an unpaired two-tailed *t*-test. $P < 0.05$ was considered to be significant.

Results

Spontaneous Dermatitis of Flg^{ft} *Mice in the Steady State under SPF Conditions*

As described previously,[14,15] the expression of the filaggrin monomer was barely detectable by Western blotting in the dorsal skin of *Flg^{ft}* mice compared with that of B6 mice (data not shown). Here, we investigated the clinical manifestations seen in the skin of *Flg^{ft}* mice raised in a steady state under SPF conditions and found that *Flg^{ft}* mice developed spontaneous dermatitis (Figure 1A). The clinical severities of skin lesions, including pruritic activity, erythema, edema, erosion, and scaling, were scored. The total clinical scores of *Flg^{ft}* mice increased with age

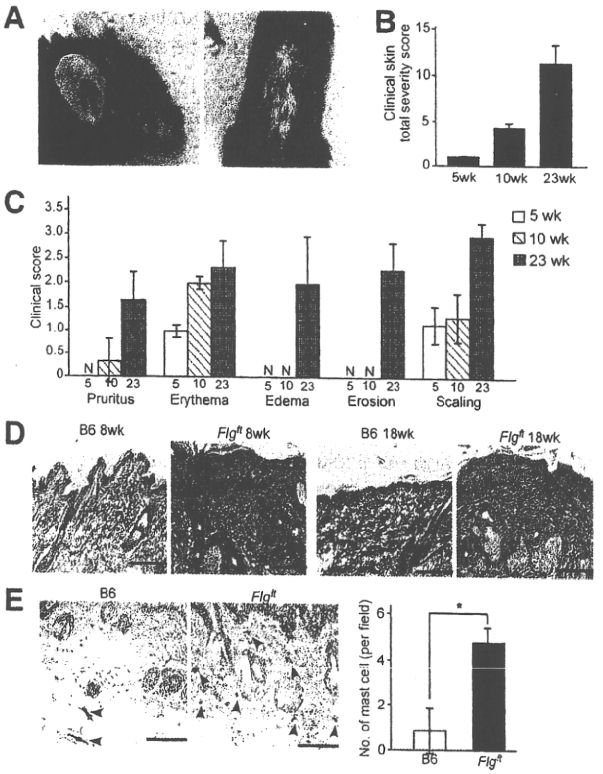

Figure 1. Spontaneous dermatitis in *Flg*^{ft} mice in SPF. **A:** Clinical photographs of 20-week-old *Flg*^{ft} mice. Total clinical severity scores (**B**) for each particular item (**C**) in 5-, 10- and 23-week-old *Flg*^{ft} mice. N, none. **D:** H&E-stained sections in 8- and 18-week-old mice. Scale bar = 100 μm. **E:** Toluidine blue staining of the skin from 8-week-old B6 and *Flg*^{ft} mice and the numbers of mast cells (**arrowheads**) per field are shown. $^*P < 0.05$.

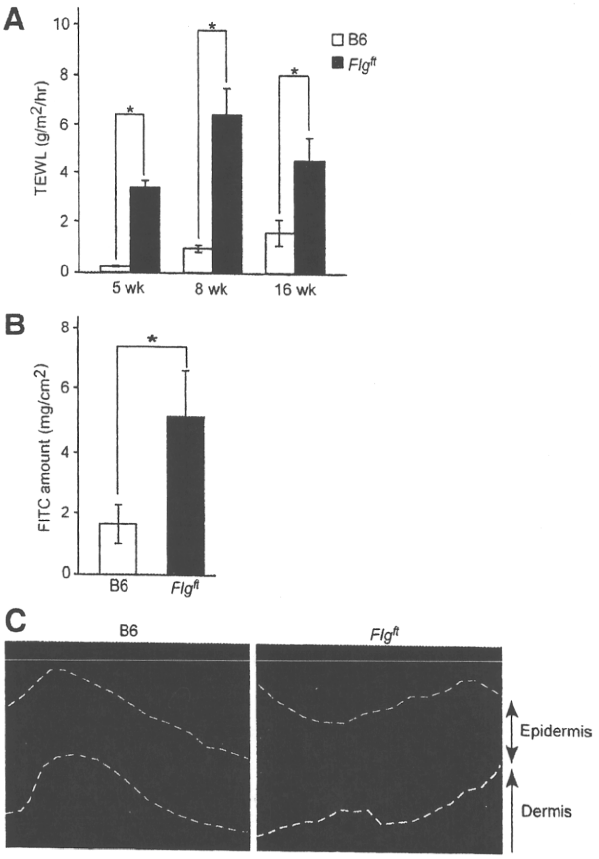

Figure 2. Skin barrier dysfunction in *Flg*^{ft} mice. **A:** TEWL through dorsal skin of 5-, 8-, and 16-week-old B6 and *Flg*^{ft} mice. **B:** Amount of FITC in the skin of B6 and *Flg*^{ft} mice after topical application. **C:** Fluorescence intensities of FITC of the skin after topical application. **Dashed white lines** indicate the border between the epidermis and the dermis, and the top of the epidermis. $^*P < 0.05$.

(Figure 1B). The first manifestations to appear when mice were young were erythema and fine scaling; pruritic activity, erosion, and edema followed later (Figure 1C). In contrast, no cutaneous manifestation was observed in either B6 mice, studied as a control, or heterozygous mice intercrossed with *Flg*^{ft} and B6 mice kept under SPF conditions throughout the experimental period (data not shown). In addition, there was no apparent difference in terms of clinical manifestations between the genders of *Flg*^{ft} mice throughout the period (data not shown).

Histological examination of skin from *Flg*^{ft} mice revealed epidermal acanthosis, increased lymphocyte infiltration, and dense fibrous bundles in the dermis in both younger (8-week-old) and older (18-week-old) *Flg*^{ft} mice; none of these were observed in B6 mice (Figure 1D). In addition, toluidine blue staining to detect mast cells showed an increased number of mast cells, especially degranulated mast cells in the upper dermis, in *Flg*^{ft} mice (Figure 1E). No mouse or human mite bodies were detected in the sections. These data support the diagnosis of spontaneous clinical dermatitis in *Flg*^{ft} mice in the steady state under SPF conditions.

Defect of Skin Barrier Function in Flg^{ft} *Mice*

Because barrier dysfunction is a common characteristic of AD,[4–7,21] we measured TEWL, an established indicator of barrier function.[21] TEWL was significantly higher in *Flg*^{ft} mice than in B6 mice from an early age (4 weeks) to an older age (16 weeks) (Figure 2A). Because TEWL is only a measure of water transportation through the skin from the inside to the outside of the body, another experimental method was necessary to evaluate outside-to-inside barrier function from the perspective of invasion of external stimuli. To address this issue, we measured FITC penetration through the skin from the outside. FITC solution was applied to the shaved dorsal skin of 8-week-old female mice; 3 hours later, the epidermis was separated and homogenized so that the FITC content could be measured with a fluorometer. The epidermis of *Flg*^{ft} mice contained a higher amount of FITC than that of B6 mice (Figure 2B). Neither group had FITC in the dermis after this procedure, however (data not shown). In addition, observation of fluorescence intensities in the epidermis of both mice showed stronger fluorescence in *Flg*^{ft} mice (Figure 2C). To further analyze the skin permeability, we examined the mouse embryos by toluidine blue solution and showed that the *Flg*^{ft} embryo was entirely dye-permeable compared with the control littermate (Supplemental Figure S1, see *http://ajp.amjpathol.org*). These data strongly indicate a defect in the skin barrier of *Flg*^{ft}