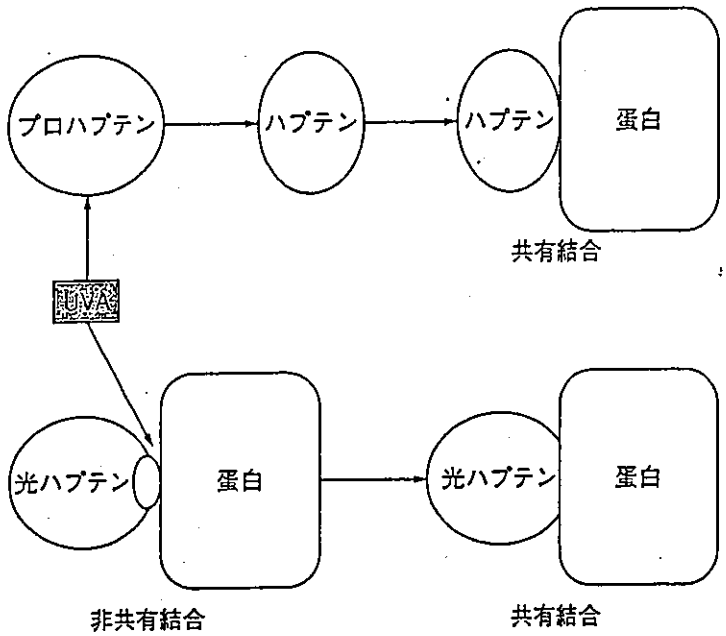

図 24-1　光ハプテンとプロハプテン

わち通常の抗原と異なり，光アレルギー性物質が抗原となるには UV 照射が必要となる．このことについては古くよりいくつかの説が提唱されてきたが，大きくは 2 つの機序に集約される．一つは光ハプテン photohapten であり，もう一つはプロハプテン prohapten である（図 24-1）．プロハプテンは UV 照射により化学構造の変化が起き，通常のハプテンと同じように蛋白との結合能力を獲得する，という単純明快な説である．一方，光ハプテン説は，UV 照射がなされるとその一部が光分解され，その分解と同時に近傍の蛋白と共有結合し完全抗原ができあがるという考えである[9]．したがって，おそらく光ハプテンと蛋白とは UV 照射の前に予め非共有結合していなければならず，UV はこれを共有結合に変えるのでろう．また物質によっては光ハプテンになるために，生体内で代謝を受ける必要があるかもしれず，プロ光ハプテン prophotohapten というべき性質のものも存在すると考えられる．

代表的な光ハプテンは TCSA であり[10]，UVA 照射により 4 つある塩素のうち一つがはずれると同時にフリーラジカルが形成され，共存する蛋白と光共有結合する．しかし予め UVA 照射した TCSA（多くは光分解産物であるトリクロロサリチルアニリドになっている）と蛋白を共存させても，両者の共有結合は認められない．さらに TCSA を塗布し UVA を照射した免疫マウスにトリクロロサリチルアニリドを塗布しても，惹起反応は起こらない．スルファニルアミドなどではプロハプテンであることが示唆されているが，光アレルギー性物質のかなりの部分は光ハプテンであろうと考えられる．事実，アレルギー性の薬剤性光線過敏症の原因物質であるアフロクァロン[11]やキノロン[5]も光ハプテンであり，ピロキシカムもこうした性質をもつが，不思議なことにアンピロキシカムはプロハプテンである．さらにサンスクリーン剤であるベンゾフェノン-3[9]も光ハプテン能を有する．プロ光ハプテンである物質の存在はまだ明らかではないが，フロタミドはこれにあたるかもしれない[12]．いずれにしろ，光ハプテン能は光アレルギー性物質の性格を検討す

る上で最も重要な点である.

光ハプテンと蛋白の光結合様式の詳細は明らかではない. しかしニューキノロンであるオフロキサシンはリジンに光結合する選択性が高く[13], おそらくアミノ基に結合することが示唆される. したがって, リジン側鎖やN末端のアミノ基に光結合しその抗原性を発揮すると考えられる.

d. 光ハプテンの細胞への光結合: 光ハプテン修飾細胞

マウスの剃毛皮膚に光ハプテンを塗布し, 同部にUVAを照射すると感作が成立し, 耳翼を光ハプテン塗布・UVA照射することにより惹起反応をみる. これはアレルギー性光接触皮膚炎のモデルである. 光ハプテン溶液に浮遊させた表皮細胞をUVA照射すると光ハプテン修飾表皮細胞が形成される. この修飾細胞をマウスの皮下に投与することにより, やはり感作, 惹起を行うことができる. 前者の経皮的な感作, 惹起と後者の光ハプテン修飾細胞を用いた感作, 惹起には互換性があり, 光ハプテン修飾細胞は光抗原を担った *in vivo* で形成される細胞の擬態ということができる (図24-2). こうした抗原性をもった光修飾細胞は, TCSA[14], アフロクァロン[11], キノロン[5], ベンゾフェノン-3[9]で作製可能であることが明らかとなっている.

Langerhans細胞 (LC) は通常の接触皮膚炎と同様に, 光接触皮膚炎においても抗原提示細胞として働く[2,3]. さらに薬剤性光線過敏症においても光抗原を提示しうる細胞として機能している[15,16]. 光ハプテンで感作したマウスより得た感作T細胞を光ハプテン化したLCと培養すると, T細胞の増殖反応が起こる. したがって光ハプテン修飾細胞は *in vitro* でも抗原性を発揮する. しかしこの *in vitro* の系では, 光ハプテンの種類によって, その光修飾細胞がT細胞を刺激しやすいものとそうでないものとがある. たとえばTCSAの場合, ただ単にTCSA光修飾LCを刺激細胞として用いただけではT細胞の増殖反応は得られず, 抗原提示細胞としてマクロファージを共存させた場合にT細胞は刺激される[14]. 光修飾LCのみでT細胞刺激を行おうとすると, 狭い範囲での適度のTCSA濃度, UVA照射量を用いた処置が必要となる. これはTCSAは光アレルギー性とともに強い光毒性を有しているため[7], TCSA光修飾表皮細胞のviabilityは非常に低くなり, 他の生きた抗原提示細胞に新たにプロセッシング, 提示されないと感作T細胞を増殖できないことによる. *in vivo* では恐らくこの再プロセッシングも起こっており, 光修飾細胞の皮下投与によって過敏症が誘導, 惹起できるのであろう. 一方キノロンをはじめとする薬剤はTCSAに比べると細胞毒性は弱く, その光修

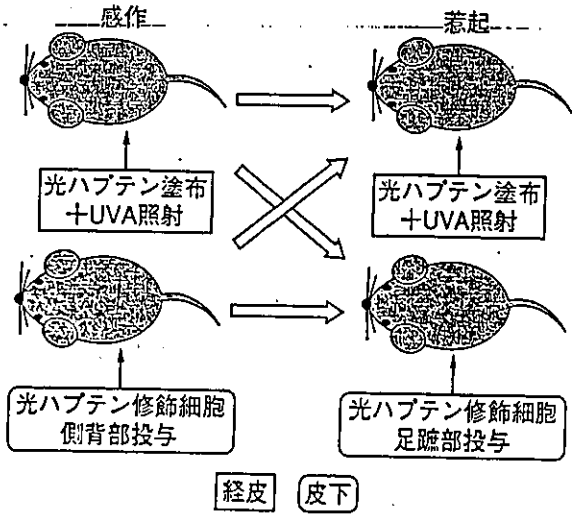

図24-2　マウスにおける光ハプテンに対する光線過敏症の感作, 惹起方法

飾表皮細胞の viability は高く保たれている[5]．したがって他の抗原提示細胞を添加しなくても *in vitro* での T 細胞増殖反応を誘導することができると考えられる[16]．

e. 光ハプテンと UVA による LC 上の光抗原形成と LC の抗原提示能促進

LC による光抗原の提示において，光ハプテンが LC 上の主要組織適合抗原複合体（MHC）クラスⅡ分子あるいはクラスⅡ分子によって表出された自己ペプチドに直接光結合するのか，あるいは UVA 照射によってできた光ハプテン-蛋白複合体が LC にいったん取り込まれ，クラスⅡ分子とともに再表出されるのかは不明である．しかし LC を *in vitro* で光ハプテン化し感作 T 細胞と培養した場合，通常の 3 日間培養で T 細胞増殖反応がみられることから，光ハプテンは直接 MHC クラスⅡ分子-自己ペプチド複合体に光共有結合すると想像される（図 24-3）．事実，キノロン光線過敏症において，クラスⅡに親和性のあるリジンを含むペプチドをキノロンと光共有結合させ，これを LC に負荷し，その抗原提示の有無を感作 T 細胞反応としてみると，T 細胞は増殖する[13]．

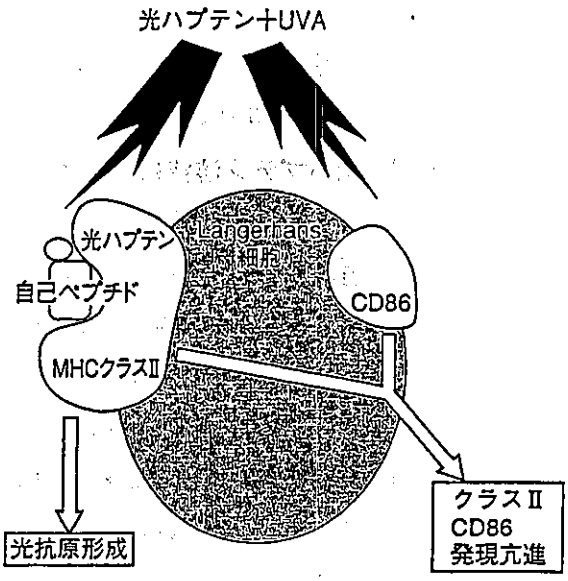

図 24-3　光ハプテンと UVA が LC に与える変化

加えてハプテンと同様に，光ハプテンも UVA 照射下で LC に作用してその抗原提示能を促進させる変化を起こす．LC を TCSA 溶液に浮遊させ UVA を照射すると，適当量では LC の MHC クラスⅡおよび CD86 発現が増す．この両表面分子の発現亢進は一部の LC に同時にみられる．また TCSA のみあるいは UVA のみではこうした LC 上の変化は起こらないため，TCSA が UVA の作用により LC の表面に共有結合した結果，シグナル伝達が起こったものと考えられる．すなわち UVA 照射下で光ハプテンは光アレルゲンとして作用するばかりではなく，LC の抗原提示にかかわる機能も亢進させる[17]．

f. 光ハプテンに対する T 細胞反応

光ハプテンに対する T 細胞の反応には当然ながら MHC 拘束性があり，また過敏症の起こりやすさは MHC クラスⅡのハプロタイプに依存している．たとえばマウスの TCSA 光線過敏症では，ハプロタイプが d，b の場合，高反応性であり，k では低反応性となる[10]．k ハプロタイプが低反応性となるのは，サプレッサー細胞である Th2 細胞が I-E 拘束性に誘導されやすいことによ

ると考えられる．あるいは現在の概念によれば調整性 T 細胞（regulatory T 細胞）に相当するかもしれない．光ハプテン修飾 LC による光抗原特異的 T 細胞の反応には，クラスⅡ分子と T 細胞受容体の結合以外に，LC 上の CD86 と T 細胞上の CD28 が co-stimulatory 分子として重要である[16]．

一つの光ハプテンが 1 クローンの T 細胞を刺激するか，複数の T 細胞クローンを増殖させるかは，光ハプテンの抗原決定基の個数や蛋白光共有結合部位の数によるだろう．マウスのキノロン反応性 T 細胞は T 細胞受容体 Vβ13 を有し[16]，TCSA 反応性 T 細胞は Vβ7 をもつ[18]．ヒトでの反応も薬剤性光線過敏症患者での末梢血単核球を用いて，一部の薬剤で確認しうる[19]．

g．光接触皮膚炎と薬剤性光線過敏症の違い

両者とも光ハプテンの投与によって起こる疾患としても，その投与経路は異なる．光接触皮膚炎では経皮的であり，薬剤性光線過敏症では経口的である．皮膚に UV が当たり表皮細胞が光ハプテン化され，その過敏症の誘導に LC が関与するのは共通であろう（図 24-4）．しかし，アレルギー性光接触皮膚炎の組織学的反応は湿疹型であるが，薬剤性光線過敏症のそれは多様であり，苔癬型組織反応をとることすらある．光ハプテンの表皮への到達経路は，光接触皮膚炎の場合は角層側からであり，薬剤性光線過敏症では基底層側からである．したがって表皮細胞の光ハプテン化の分布には両者間で差が生じることになる．こうした密度勾配が組織反応の違いを生ずるかもしれない．両者とも光ハプテン特異的 CD4 陽性細胞が過敏症発症にかかわるが，病変誘発には

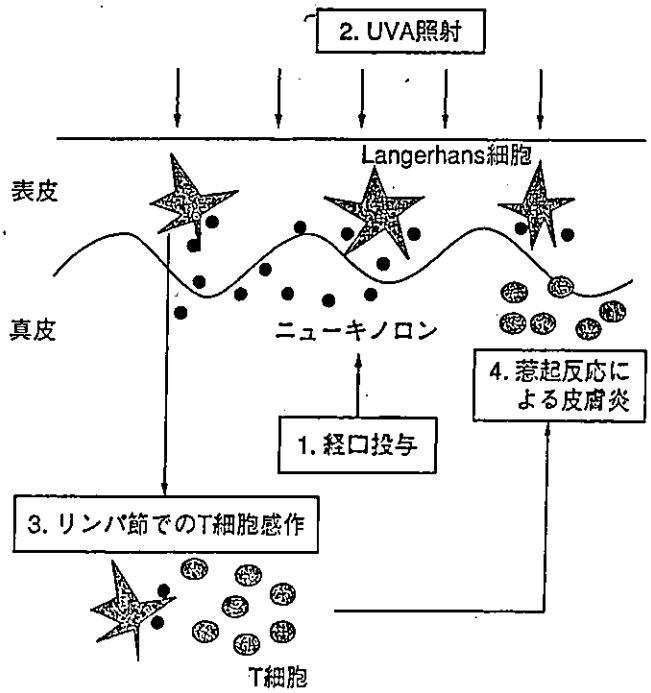

図 24-4　薬剤性光線過敏症の発症機序

CD8 陽性の参加も重要であろう．この CD8 陽性 T 細胞関与の軽重も表皮細胞での光抗原の密度勾配によって生ずるかもしれない．

h. 自己免疫性光線過敏症：光抗原によらない光線過敏症の存在

慢性光線過敏性皮膚炎 chronic actinic dermatitis (CAD) はそれまで，呼称されていたいくつかの光線過敏症の名称を統合する形で生まれた疾患概念であり，基本的に慢性であって原因が不明の疾患を念頭にこう呼称している[20]．persistent light reaction，actinic reticuloid もこの疾患概念に含まれ，光抗原の投与なくして持続的に光線過敏症を引き起こす状態を指し，患者自身を persistent light reactor とよんでいる．persistent light reactor の中にはある物質に光貼布試験陽性を示す患者がおり，光線過敏症は以前その物質に対する光接触皮膚炎であったものが，光アレルゲンなしに UV に感受性をもつようになってしまった状態と解される．しかしその機序はいまだ明瞭ではなく，光感受性物質なしに UV が自己反応性 T 細胞を何らかの機序で活性化させてしまう可能性，また古典的には光感受性物質が微量に皮膚に残っている可能性，など考えられている．

いずれにしても，こうした光線過敏症は光ハプテンやプロハプテンによらないアレルギー性光線過敏症が存在することを示唆している．UV 照射がいかにこうした過敏症のもとになる自己抗原 (?) の修飾を行うか，あるいはアジュバント効果を発揮するのか，またそもそもの過敏症を引き起こした光抗原反応性 T 細胞と自己反応性 T 細胞にはどんな関係があるのかは不明であり，今後解明されなければならない問題である．

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〈戸倉新樹〉

Chronic actinic dermatitis associated with adult T-cell leukemia

Kazunari Sugita, MD, Takatoshi Shimauchi, MD, and Yoshiki Tokura, MD
Kitakyushu, Japan

We describe a patient with chronic actinic dermatitis that occurred with the progress of adult T-cell leukemia. Immunohistochemically, $CD8^+$ T cells, but not $CD4^+$ cells, predominantly infiltrated the lichenoid lesional skin, indicating that the eruption was induced by reactive, normal $CD8^+$ T cells but not adult T-cell leukemia cells. Our patient suggests that chronic actinic dermatitis may occur in association with the advanced human T-lymphotrophic virus-I infectious disorder. (J Am Acad Dermatol 2005;52: S38-40.)

Chronic actinic dermatitis (CAD) is persistent light reactivity encompassing actinic reticuloid and photosensitive eczema.[1] Various skin eruptions have been reported in adult T-cell leukemia/lymphoma (ATL),[2] in which tumor cells are mostly $CD4^+CD25^+$ T-lymphocytes with various cytokine expression/production patterns.[3] Although the association between CAD and T-cell lymphoma has been reported for more than 15 years,[4] photosensitivity occurring in association with ATL has not been described. We report a case of CAD associated with ATL.

A 76-year-old retired male laborer presented with a 2-month history of pruritic dermatitis on sun-exposed sites. His medical history included angina pectoris and mild leukocytosis that had been occasionally indicated over 20 years. He had been taking only a Chinese medicine for 3 years. Physical examination revealed erythematous plaques with scaly papules on the sun-exposed areas (Fig 1). The peripheral blood showed normal counts of leukocytes (7200/μL) with normal differentiation. Phototesting disclosed that his minimal erythema dose of UVB was 10 mJ/cm^2 (normal, 50-150 mJ/cm^2), and UVA abnormally induced erythema at a minimal dose of 3.6 J/cm^2.

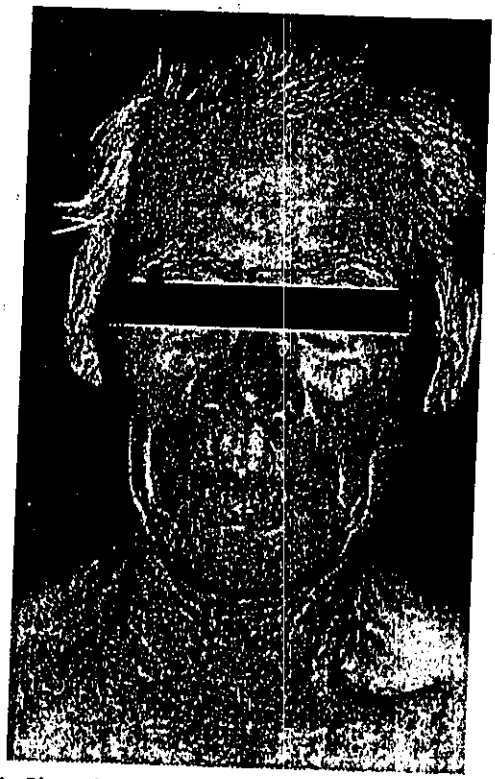

Fig 1. Photodistributed erythematous eruption on face.

Photopatch testing was negative for his medication. A skin biopsy specimen showed a bandlike infiltrate of small lymphocytes, with liquefaction degeneration of the basal epidermis (Fig 2, *A*). The infiltrating lymphocytes were predominantly $CD8^+$ (Fig 2, *B*). The patient stopped taking his medication and was treated with topical corticosteroids. Two

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From the Department of Dermatology, School of Medicine, University of Occupational and Environmental Health.

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Reprint requests: Kazunari Sugita, MD, Department of Dermatology, School of Medicine, University of Occupational and Environmental Health, Japan, 1-1 Iseigaoka, Yahatanishi-ku, Kitakyushu 807-8555, Japan. E-mail: k-sugita@med.uoeh-u.ac.jp.

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ulcers.[18] In addition to corticosteroids, TNF-α inhibitors have shown potential benefit in the management of aphthous ulcers. Thalidomide has been documented to inhibit TNF-α production and to diminish the stability of its messenger RNA.[19] Furthermore, a recent case report documents the successful treatment of refractory, recurrent aphthous ulcers in a 50-year-old woman with etanercept, a soluble fusion TNF-α receptor protein.[20] The main mechanism of etanercept involves the blocking of TNF-α binding to its cell surface receptor, thereby diminishing the proinflammatory T_H1 responses.

The antagonistic effects of etanercept and imiquimod on TNF-α underscores a potential link in the immunopathology that may lead to the development of aphthouslike ulcers after the topical application of imiquimod. Our 3 cases illuminate another possible side effect of the use of imiquimod in sun-damaged skin particularly of the vermilion: the appearance of aphthous ulcers. Although none of our patients opted for a medication rechallenge, we believe that the temporal association with the use of imiquimod and the development of aphthouslike ulcers in each of these cases is very suggestive. Furthermore, the imiquimod application creates a localized immunologic response and, therefore, the ulcerations do not have to be directly under the site of application but in the vicinity. The evidence to date showing that imiquimod induces production of some of the very cytokines found to be expressed in high levels in aphthous ulcers, makes this association to be more likely than mere coincidence.

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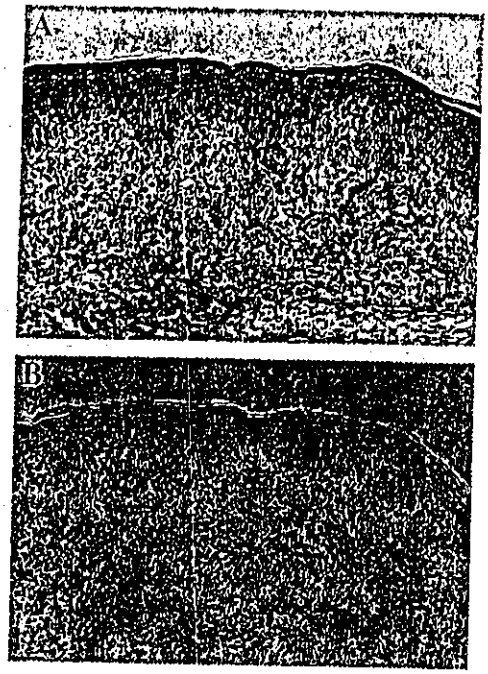

Fig 2. **A**, Histology showing lichenoid tissue reaction. **B**, Dense subepidermal bandlike infiltrate of T cells expressing CD8. (**A**, Hematoxylin-eosin stain; **B**, CD8 stain; original magnifications: **A**, ×100; **B**, ×100.)

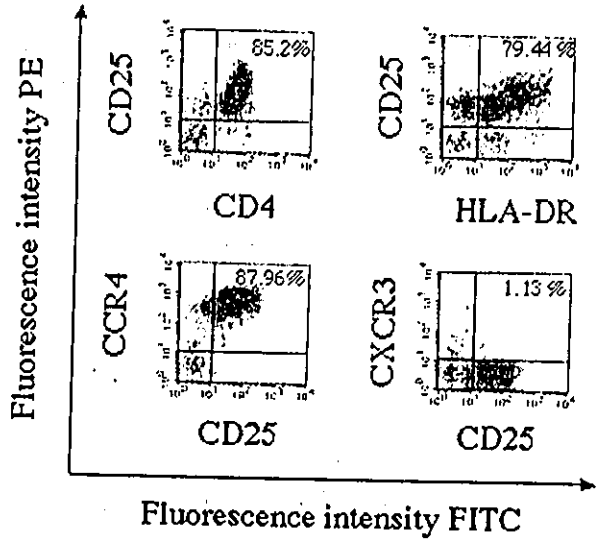

Fig 3. Flow cytometric analysis of patient's peripheral blood mononuclear cells, showing that circulating adult T-cell leukemia cells are positive for CD4 and CD25 (*top, left*), HLA-DR (*top, right*), and CCR4 (*bottom, left*), but not CXCR3 (*bottom, right*).

Table I. Cytokine profiles of patient's PBMC and normal subjects' $CD4^+$ cells in stimulation with anti-CD3/CD28 mAbs

	Stimulants	IFN-γ (pg/ml)	TNF-α (pg/ml)	IL-2 (pg/ml)	IL-4 (pg/ml)	IL-5 (pg/ml)	IL-10 (pg/ml)
Patient	—	179.5	Undetectable	Undetectable	38	16	45
	α-CD3/CD28 mAbs	4030	29	32	52	17	60
Normal healthy donors (n=5, mean ± SD)	—	1575 ± 793	353 ± 127	474 ± 284	1152 ± 422	295 ± 81	550 ± 245
	α-CD3/CD28 mAbs	10600 ± 1420	319 ± 45	662 ± 181	1185 ± 296	512 ± 321	636 ± 131

Normal $CD4^+$ cells were purified from PBMC of 5 healthy adults with anti-CD4 mAb-conjugated magnetic beads (Dynal Inc, Oslo, Norway) and DETACHaBEAD (Dynal Inc) according to the manufacturer's directions. The minimal detection levels of cytokines were as follows: IL-2, 2.6 pg/ml; IL-4, 2.6 pg/ml; IL-5, 2.4 pg/ml; IL-10, 2.8 pg/ml; TNF-α, 2.8 pg/ml; and IFN-γ, 7.1 pg/ml. The values represent the mean of duplicate cultures.

months later, however, the eruption was worsened and, therefore, we diagnosed his photosensitivity as CAD.

Concomitantly, his superficial lymph nodes were enlarged. A laboratory study revealed a leukocyte count of 15,300/μL, with 41% atypical flowerlike lymphocytes. Human T-lymphotrophic virus (HTLV)-I monoclonal integration was present. A flow cytometric analysis of peripheral blood mononuclear cells showed that approximately 85% of lymphocytes were positive for CD4, CD25, and HLA-DR and expressed Th2 chemokine receptor CCR4 but not Th1 receptor CXCR3 (Fig 3). $CD25^-$; normal $CD4^+$ T cells were less than 3%. He was given the diagnosis of ATL, acute type, based on the classification of Shimoyama.[5]

To test the functional ability of the patient's ATL cells, the amounts of cytokines listed in Table I were measured in the culture supernatants of purified ATL cells using cytometric beads array kits. Despite the expression of Th2 type chemokine receptor CCR4, neither of Th2 cytokines (IL-4, IL-5, and IL-10) were secreted by the patient's peripheral blood mononuclear cells compared with normal $CD4^+$ cells (Table I). Interferon alfa production was enhanced by anti-CD3 and -CD28 mAbs, but to a lesser degree

than the control subjects. Thus, the patient's ATL cells were not functional in the cytokine production. In our patient, $CD8^+$ T cells infiltrated in a lichenoid fashion.

Cytotoxic $CD8^+$ T cells are thought to be activated with evolution of HTLV-I infection.[6] It is possible that HTLV-I–induced activation of $CD8^+$ T cells attack epidermal cells on sun exposure. In this scenario, UV irradiation may stimulate keratinocytes to express autoantigen or autoantigens for $CD8^+$ cells. As demonstrated in most ATL cases,[7] the tumor cells in our patients expressed Th2 chemokine receptor CCR4. HTLV-1–infected $CCR4^+$ T cells have growth advantages by deviating host immune response to Th2,[7] because Th2 cytokines suppress $CD8^+$ tumoricidal T cells. In this case, however, the production of Th2 cytokines by HTLV-1–infected T cells was greatly reduced, and moreover, normal T cells scarcely existed. This may allow $CD8^+$ T cells to be activated, resulting in the development of the skin eruption. Recently, CAD has been reported in patients with HIV infection.[8-10] In these patients, the abnormal response to UVB radiation and lichenoid tissue reaction seem to be hallmarks of the advanced disease.[11] We note striking similarities in photosensitivity dermatitis between patients with HIV infections and this case. In our patient, $CD4^+$ tumor cells were functionally impaired in the production of cytokines, and normal $CD4^+$ T cells were very low in number. This immunologic state is virtually the same as that seen in AIDS. Our findings may provide a new clue to elucidate the pathogenesis of CAD from the view of immune dysfunction.

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Regular Paper

Analysis of 8-Hydroxyguanine (8-OH-Gua) Released from DNA by the Formamidopyrimidine DNA Glycosylase (Fpg) Protein: A Reliable Method to Estimate Cellular Oxidative Stress

Hiroshi ORIMO[1,4], Nan MEI[2], Serge BOITEUX[3], Yoshiki TOKURA[4] and Hiroshi KASAI[1]*

8-OH-Gua background level/Fpg protein/γ-irradiation.

To improve the analyses of a form of oxidative DNA damage, 8-hydroxyguanine (8-OH-Gua), we treated isolated DNA with formamidopyrimidine DNA glycosylase (Fpg) and analyzed the released 8-OH-Gua by using a high-performance liquid chromatography system equipped with an electrochemical detector (HPLC-ECD). The human lung carcinoma cells (A549) and human keratinocyte (HaCaT) were irradiated with γ-rays. After the isolated DNA was treated with the Fpg protein, we analyzed the released 8-OH-Gua by using an HPLC-ECD. With this method, the background level of 8-OH-Gua in DNA from human lung carcinoma cells was determined to be 3.4 residues per 10^7 guanine (Gua). A similar background level of 8-OH-Gua (3.1 residues per 10^7 Gua) was also detected in human keratinocyte DNA with this method. These background 8-OH-Gua levels in cellular DNA are comparable to that obtained previously by an analysis of 8-OH-dGMP after nuclease P1 digestion of cellular DNA (4.3 residues per 10^7 dCMP). A dose-dependent increase of 8-OH-Gua ($0.17/10^7$ Gua/Gy) was observed after cells were irradiated with γ-rays. Twenty hours after γ-irradiation with 60 Gy, 75% of the 8-OH-Gua produced in keratinocyte DNA was repaired. With our new analysis method, it is possible to detect the small changes in the 8-OH-Gua levels in cellular DNA induced by various environmental factors.

INTRODUCTION

The formation of 8-hydroxyguanine (8-OH-Gua) in DNA by reactive oxygen species (ROS) was first reported in 1984.[1)] The 8-OH-Gua is a major product of DNA damage induced by the reactions of reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), superoxide anions (O_2^-), singlet oxygen, and hydroxyl radicals (· OH).[2)] It is also called 7,8-dihydro-8-oxoguanine (8-oxo-Gua).[1)] It is used as a biomarker of oxidative DNA damage[3–4)] and causes mainly GC to TA transversions in mammalian cells.[5)] Floyd *et al.*[6)] introduced a highly sensitive analytical method for 8-OH-Gua, which uses high-performance liquid chromatography (HPLC) coupled to an electrochemical detector (ECD). DNA isolation methods have also improved the analysis, and the modified chaotropic sodium iodide (NaI) technique yields the lowest and least variable 8-OH-Gua values.[7,8)]

This report describes an assay to detect 8-OH-Gua after cellular DNA is treated with the formamidopyrimidine DNA glycosylase (Fpg) enzyme following DNA extraction.[9–11)] In this assay, we used the biological function of the Fpg protein to remove the 8-OH-Gua that is formed in ds-DNA.[9–11)] In the present study, two different cell lines, A549 (lung carcinoma) and HaCaT (keratinocyte), were used, since these cell lines from an internal organ and skin may have different defense abilities against ROS and different repair responses. This method reduced the background level of 8-OH-Gua in DNA and improved the reliability once the increase in oxidative DNA damage in these γ-irradiated human cells was measured.

MATERIALS AND METHODS

Cells and culture conditions

Human alveolar epithelial cells (A549), originally derived from an individual with alveolar cell carcinoma, were pur-

*Corresponding auther: Phone: +81-93-691-7469,
Fax: +81-93-601-2199.
E-mail: h-kasai@med.uoeh-u.ac.jp
[1]Department of Environmental Oncology, Institute of Industrial Ecological Sciences, University of Occupational and Environmental Health, Kitakyushu, 807-8555 Japan.
[2]Division of Genetic and Reproductive Toxicology, National Center for Toxicological Research, FDA, Jefferson, AR 72079, U.S.A.
[3]Commissariat àl'Energie Atomique (CEA), Direction des Sciences du Vivant (DSV), Département de Radiobiologie et Radiopathologie, Unité Mixte de Recherche (UMR) 217 CNRS-CEA Radiobiologie Moléculaire et Cellulaire, 92265 Fontenay aux Roses, France.
[4]Department of Dermatology, University of Occupational and Environmental Health, Kitakyushu, 807-8555 Japan.

chased from the American Type Culture Collection (Rockville, MD, USA). This cell line was cultured in Eagle's Minimum Essential Medium with kanamycin (Nissui Pharmaceutical, Tokyo, Japan), supplemented with 10% fetal bovine serum, L-glutamine (2 mM), and nonessential amino acids (0.1 mM), and was maintained at 37°C in a humidified atmosphere containing 5% CO_2 in air. Unless otherwise noted, all culture supplies were purchased from Gibco-Invitrogen (Carlsbad, CA, USA).

The human keratinocyte (HaCaT) cell line[12] was cultured in Dulbecco's Modified Eagle's Medium (Nissui Pharmaceutical, Tokyo, Japan), supplemented with 10% fetal bovine serum, L-glutamine (2 mM), 100 units/ml penicillin, 100 µg/ml streptomycin sulfate, and sodium pyruvate (1 mM) and was maintained at 37°C in a humidified atmosphere containing 5% CO_2 in air.

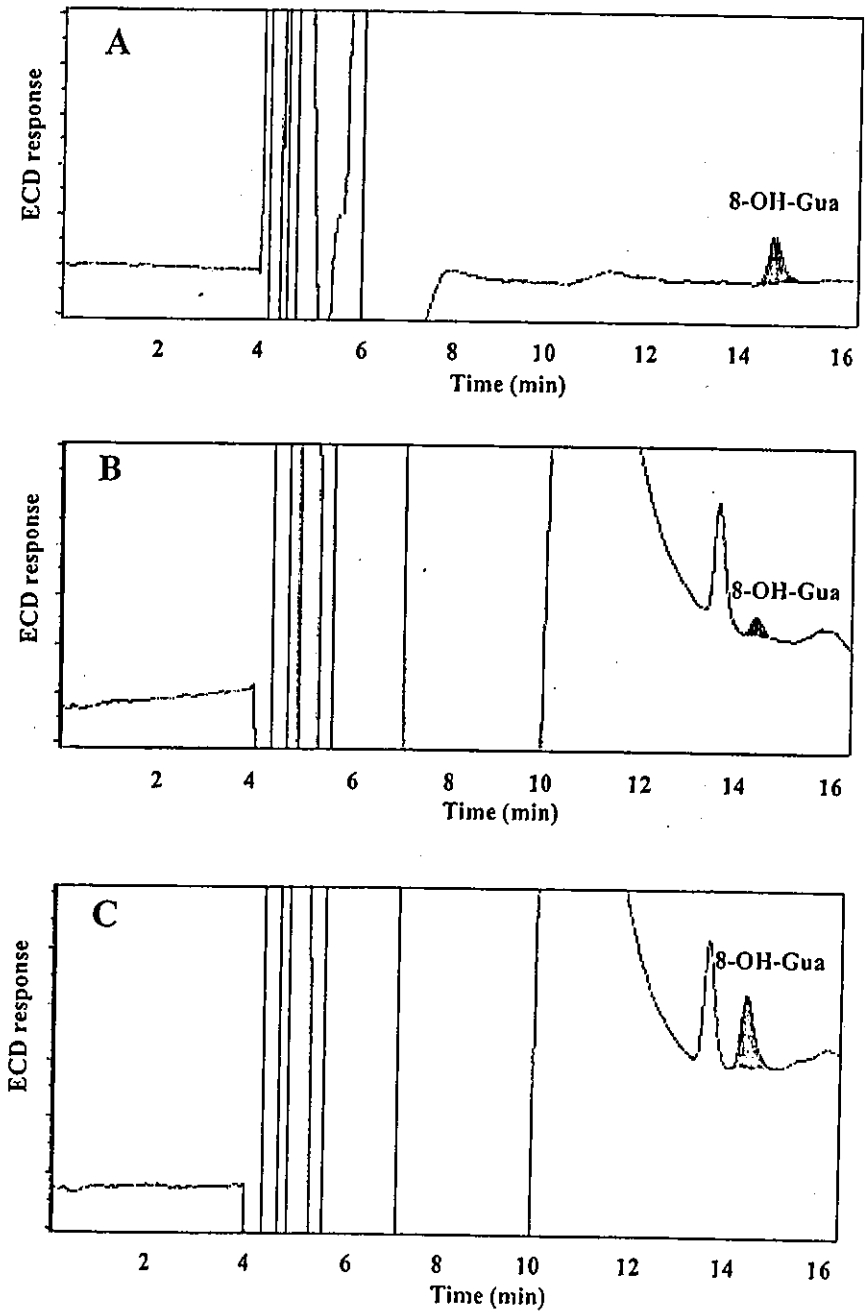

Fig. 1. Typical example of 8-OH-Gua analysis. DNA isolated from A549 cells was treated with the Fpg protein, as described in the Materials and Methods section, and 70 µl portions of the samples were analyzed by an HPLC-ECD. (A) Authentic 8-OH-Gua (5.6 pg). (B) DNA from unirradiated cells. (C) DNA from 80 Gy γ-irradiated cells.

Irradiation of cells with γ-rays

The cells were seeded into 100 mm diameter tissue culture dishes and allowed to attach for a period of 16–24 hours at 37°C. The cells were then irradiated on ice with a gamma cell irradiator (GE40E; Nordion, Ottawa, Canada) at varying doses (dose rate, 0.96 Gy/min). Control cells were not irradiated. After irradiation, the cells were harvested, washed with ice-cold phosphate-buffered saline, and immediately processed to a cell viability test and DNA isolation. Cell viability was determined by a trypan blue dye-exclusion test (0.4%) (GIBCO-BRL, Grand Island, NY, USA).[13]

Determination of 8-OH-Gua in cellular DNA

Cellular DNA was isolated by using a DNA extractor WB kit (Wako, Osaka, Japan).[7,14] Desferal (deferoxamine mesylate; Sigma, St. Louis, MO, USA) was added to the lysis solution (1 mM) to prevent DNA oxidation.[8] The isolated DNA (50–150 μg) was dissolved in 200 μl distilled water, and its concentration was measured with a UV spectrophotometer (JASCO V-520). The DNA solution (150 μl) was mixed with 15 μl of buffer (0.5 M Tris-HCl, 0.5 M KCl, and 20 mM EDTA; pH 8.0) and bovine serum albumin (final concentration, 90 μg/ml), and it was treated with the Fpg protein (10 μg /sample) at 37°C for 20 min.[15] This solution was filtered with an Ultrafree-Probind filter (Millipore, Bedford, MA, USA), and a 70 μl aliquot of the sample was injected into an HPLC column (YMC-Pack ODS-AM, 5 μm, 4.6 × 300 mm; temperature, 25°C; flow rate, 0.7 ml/min) equipped with an ECD (Coulochem II; ESA Inc., USA; electrode 1, 150 mV; electrode 2, 300 mV; guard cell, 350 mV). The mobile phase consisted of 10 mM sodium dihydrogenphosphate dihydrate containing 3% methanol. As the standard sample, 80 μl of an 8-OH-Gua solution (70 pg/ml) was injected. To obtain the standard solution, 8-OH-Gua (2 mg) was completely mixed with 1 liter of deionized water for 30 min at room temperature, then centrifuged (at 800 *g*, 5 min). The concentrations of 8-OH-Gua were calculated based on its UV absorption extinction coefficient value.[16] The digested DNA and the standard samples showed 8-OH-Gua peaks within the limits of detection. The 8-OH-Gua level in the DNA was expressed as the number of 8-OH-Gua per 10^7 Gua, by assuming that 20 O.D. units of DNA is 1 mg[17] and that the GC content of human DNA is 41%.[18]

Statistical analysis

Analyses were performed by use of the StatView-J 5.0 program (Berkeley, CA, USA). All data were expressed as the mean ± standard deviation (SD) from 3–8 independent measurements. Statistical significance was determined by the one-way analysis of variance (ANOVA), followed by the Scheffe test, using $P < 0.05$ as the level of significance.

RESULTS

Analysis of 8-OH-Gua in γ-irradiated A549 cells

We first optimized the conditions for the release of 8-OH-Gua from DNA by Fpg treatment. DNA samples from A549 cells were incubated with various amounts of the Fpg protein for different times. The amount of 8-OH-Gua released from DNA reached a plateau above 5 μg/tube Fpg concentration (20 min incubation time). Time-course experiments showed that the release of 8-OH-Gua became a plateau after a 20 min incubation (with 10 μg Fpg). Therefore we chose assay conditions of 10 μg/tube Fpg concentration and 20 min incubation time. A typical example of 8-OH-Gua analysis is shown in Fig. 1. The detection limit of 8-OH-Gua was about 0.5 pg. In the DNA of untreated A549 cells, the 8-OH-Gua level was measured as 3.35 ± 1.67 (SD) per 10^7 Gua (Fig. 2). Irradiation by γ-rays (20–80 Gy) induced the formation of 8-OH-Gua in a dose-dependent manner, and its yield was estimated to be approximately 0.17 residues per 10^7 Gua per Gy. Immediately after the 80 Gy γ-irradiation, the cell viability was virtually the same as that of the control, as monitored by trypan blue staining.

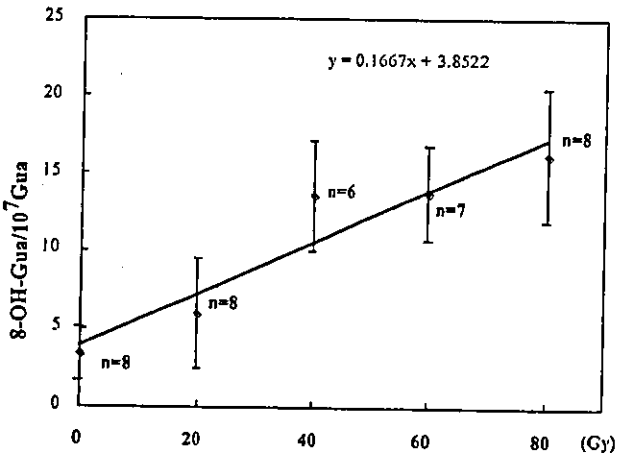

Fig. 2. Effect of γ-irradiation on the 8-OH-Gua formation in A549 cells. A549 cells were exposed to γ-irradiation at doses of 20–80 Gy. The 8-OH-Gua levels in the isolated DNA were determined as described. The results represent the means ± SD of 6–8 experiments.
Correlation (y = 0.1667x + 3.8522) *$P < 0.0001$

Analysis of 8-OH-Gua in γ-irradiated HaCaT cells

We also tried this new approach for 8-OH-Gua analysis in keratinocyte (HaCaT cells) DNA. In the DNA of untreated HaCaT cells, the 8-OH-Gua level was measured as 3.14 ± 1.94 (SD) per 10^7 Gua (Fig. 3). The formation of 8-OH-Gua was dependent on the γ-ray dose, and the yield was 0.18 residues per 10^7 Gua per Gy. Immediately after the γ-irradiation with 80 Gy, the cell viability was unchanged compared to

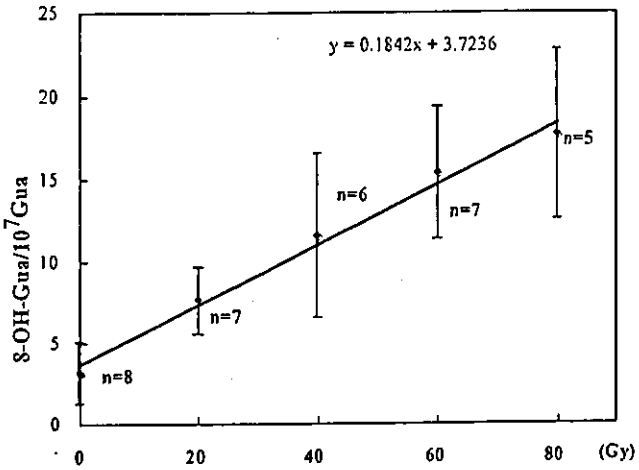

Fig. 3. Effect of γ-irradiation on 8-OH-Gua formation in HaCaT cells. HaCaT cells were exposed to γ-irradiation at doses of 20–80 Gy. The results represent the means ± SD of 5–8 experiments. Correlation (y = 0.1842x + 3.7236) $^*P < 0.0001$.

that of the control, as assessed by trypan blue dye-exclusion test.

8-OH-Gua repair in HaCaT cell DNA after γ-irradiation

We also compared the 8-OH-Gua levels in keratinocyte (HaCaT cells) DNA immediately after and 20 hours after 60 Gy γ-irradiation (Fig. 4). We found that 75% of the 8-OH-Gua formed by the γ-irradiation was repaired during the 20 hour period. Immediately after the 60 Gy γ-irradiation, the cell viability was similar to that of the control. Therefore the level of 8-OH-Gua determined by this analysis may actually be produced in living cells.

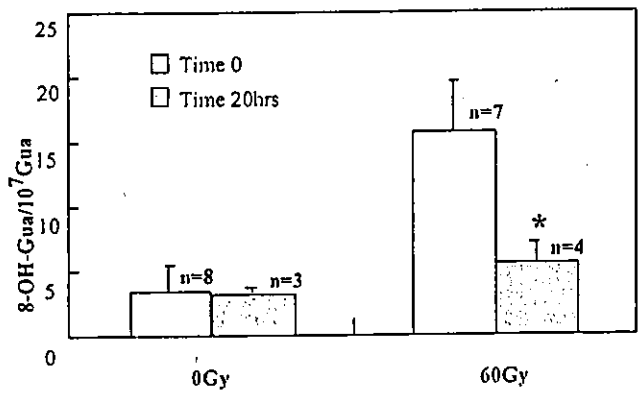

Fig. 4. The 8-OH-Gua levels in HaCaT cells immediately after and 20 hours after 60 Gy γ-irradiation (right figure). The results represent the means ± SD of 3–8 experiments. Results without γ-irradiation (0 Gy) are shown on the left.
$^*P = 0.001$ (significantly lower than the group analyzed immediately after 60 Gy γ-irradiation).

DISCUSSION

Several techniques have been developed to detect 8-OH-Gua, a type of oxidative DNA damage. The measured background levels of 8-OH-Gua differ, depending on the DNA isolation technique and the 8-OH-Gua analysis method. To measure the steady-state level of DNA oxidation, HPLC-ECD is particularly useful with its selectivity, sensitivity, and ease of quantification. During the past two decades, with improved DNA isolation techniques and enhanced HPLC-ECD sensitivity, the assayed background levels of 8-OH-Gua have decreased considerably.[7] Reliable and reproducible data with low background 8-OH-Gua levels (4/10^7 Gua) have been obtained by analyzing 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-OH-dG) after digesting DNA prepared by an improved method, using a commercially available kit, that employs an iron chelator, desferal, in the lysis step.[8] In this study we analyzed the 8-OH-Gua base by HPLC-ECD after the DNA was treated with the Fpg protein. This method was first described by Loft and collaborators.[15] They analyzed 8-nitroguanine and 8-OH-Gua in DNA after treatment with NO-generating agents, mainly in vitro, but detailed data on the background level of 8-OH-Gua in cellular DNA were not reported. Beckman *et al.* also used the same method to detect an increase of 8-OH-Gua in the DNA of H_2O_2 treated cells.[19] Karakaya *et al.*[20] detected 8-OH-Gua and many oxidized bases with a gas chromatography/isotope-dilution mass spectrometry (GC/IDMS) method, after γ-irradiated DNA was treated with the Fpg protein. In the present study, we confirmed that the background level of 8-OH-Gua in cellular DNA is 3–4 residues per 10^7 Gua. This baseline 8-OH-Gua value closely resembles that obtained by our previous method, which analyzed 8-hydroxydeoxyguanosine 5'-monophosphate (8-OH-dGMP) after the cellular DNA was digested by nuclease P1 only (4.3 8-OH-dGMP/10^7 deoxycytidine monophosphate [dCMP]).[21] We also detected a similar background level of 8-OH-Gua in the human keratinocyte DNA. In human keratinocytes, the 8-OH-Gua levels have never been measured with this level of precision. It was recently reported that relatively low doses of ultraviolet-B (62.5–500 mJ per cm^2) caused dose-dependent increases in 8-OH-dG.[22] Furthermore, that study found that unirradiated, normal, human epidermal keratinocyte DNA contained 1.49 (± 0.11) 8-OH-dG residues per 10^6 dG. In our study, the amount of 8-OH-Gua in unirradiated cell DNA was much smaller, about 1/5 of their level, though it is possible that different keratinocyte cell lines have distinct background 8-OH-Gua levels.

The Fpg protein creates single-strand breaks in the DNA at the sites of altered purines, including 8-OH-Gua. The comet assay (single cell alkaline gel electrophoresis) measures DNA breaks, which form a comet-like image in which the ratio of DNA in the tail reflects the break frequency. The

ESCODD (European Standards Committee on Oxidative DNA Damage)[23] reported that the background level of 8-OH-Gua in HeLa cell DNA is 0.53 per 10^6 guanines, based on the comet assay after Fpg treatment. Our method produced a lower background level than that of the comet assay, which is known as a highly sensitive method to measure DNA damage. They reported that the bulk of the Fpg-sensitive sites generated by a photoexcited photosensitizer (Ro 19-8022) was quite likely to be 8-OH-Gua lesions, but 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyGua) and 4,6-diamino-5-formamidopyrimidine (FapyAde), which are probably present at lower levels, might also be recognized by the Fpg protein.[23] The difference in the background levels between our analysis and the comet assay may correspond to the amounts of FapyGua and FapyAde and other unknown oxidized products, though it may also be a consequence of using different cell lines.

With our new analysis method, it is possible to detect the small changes in the 8-OH-Gua levels in cellular DNA induced by various environmental factors, such as ionizing radiation, UV light, and chemicals. Because of the low background, we could accurately analyze the reduction of 8-OH-Gua during the 20 hours after γ-irradiation (Fig. 4). Enhanced measurement precision may also contribute to the elucidation of DNA repair mechanisms. Further efforts to improve DNA isolation and analysis methods for 8-OH-Gua assays should be continued.

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Skin as an immunological organ

Yoshiki Tokura, M.D.
Department of Dermatology
University of Occupational and Environmental Health

1. Skin-associated lymphoid tissue

Since skin is an organ continuously exposed to the external stimuli, one can easily estimate the skin to be an immunologic organ. This led to the concept of skin-associated lymphoid tissue (SALT). Such organs that contact the external world include gut-associated lymphoid tissue (GALT) and bronchus-associated lymphoid tissue (BALT) as well as SALT.

The constituents of SALT include Langerhans cells, keratinocytes, skin-recruiting T cells, vascular endothelial cells, and additionally dendritic epidermal γδ T cells or DETC in mice. These cells are orchestrated to give a well-organized immune system specialized for the primary defense system.

2. Langerhans cells (LC)

Langerhans cells are bone-marrow-derived hematologic cells with a professional antigen-presenting cell ability. This dendritic cell resides in the epidermis of the skin and surrounded by keratinocytes. Thus, LC live harmoniously with the epithelial cells. The adhesion of LC to keratinocytes is performed via E-cadherin expressed on both cells. Upon external stimuli, keratinocytes produce IL-1α and TNFα, which reduces the expression of E-cadherin on LC, allowing LC to migrate to draining lymph nodes.

As antigen-presenting cells, LC present conventional protein antigens (peptides), superantigens, and haptens to T cells. This presentation is carried out with major histocompatibility complex (MHC) that binds to antigens. T cell receptors on T cells recognize antigens in the context of MHC. In addition to MHC molecules, costimulatory molecules on LC, such as CD80, CD86, CD40 and CD54, play an important role for T

Reprint requests to: Yoshiki Tokura, MD, Department of Dermatology, University of Occupational and Environmental Health, 1-1 Iseigaoka, Yahatanishi-ku, Kitakyushu 807-8555, Japan

cell activation.

The functions of LC are modified or abrogated by various stimuli. Among them, ultraviolet (UV) light is a well studied modality, which alters the antigen-presenting ability of LC. Therefore, LC has been a target in investigation of UVB-induced immunosuppression or photoimmunology.

3. Keratinocytes

More than 90% of cells in the epidermis are keratinocytes, and this epitherlial cell had been considered for long time as merely cornified, barrier-structuring cells. In early 1980's, however, keratinocytes were found to produce IL-1α. This put keratinocytes forward as immunocompetent cells and triggered discoveries of various cytokines and chamokines, such as TNF-α, GM-CSF, IL-6, IL-8, IL-10 (mice), RANTES, IP-10, Mig, I-TACK, TARC, MDC, C-TACK and etc. These molecules deeply participate in inflammation and T cell infiltration in the skin.

In another line of study, it was found that keratinocytes express MHC class II molecules, i.e. antigen-presenting molecules. Upon bearing MHC class II, keratinocytes are capable of presenting superantigens but not other antigens. Therefore, keratinocytes are a non-professional antigen-presenting cell. In the case of hapten-presentation, even immunological tolerance may be induced by class II+ keratinocytes.

Furthermore, keratinocytes express ICAM-1 or CD54 molecules when stimulated with interferon-γ. Since ICAM-1 is an adhesion molecule that binds to LFA-1 on T cells, this finding further provides evidence for immunocompetency of keratinocytes. It should be strengthened that interferon-γ released by T cells stimulates keratinocytes to express both MHC class II and CD54. Therefore, keratinocytes and T cells are mutually stimulated to evoke immunogical or inflammatory conditions.

4. Photoimmunology, photodermatology, and immunodermatology

The skin milieu is the primary target of UV light. Thus, there are close relationship among dermatology, photobiology, and immunology. When investigators in these fields study the biological effects of UV, they may need to understand the skin immune system so that accumulation of basic knowledge effectively solves clinical issues.

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◆特集／光線過敏性皮膚疾患の検査・診断と治療

薬剤性光線過敏症

戸倉新樹*

Key words：薬剤性光線過敏症(drug photosensitivity)，光線過敏型薬疹(photosensitive drug eruption)，キノロン(quinolone)，光ハプテン(photohapten)，光アレルギー(photoallergy)

Abstract 薬剤性光線過敏症は光線過敏型薬疹とも呼ばれ，成人の光線過敏症では最も多い．薬剤内服と紫外線照射が相まって発症する薬疹の特殊なタイプである．作用波長は多くの場合，UVAである．その機序は光毒性と光アレルギー性があるが，光アレルギー性機序で起こる場合が多い．薬剤の流行り廃れがあり，原因薬剤の頻度は数年から10年単位で変化する．光アレルギー性機序は，内服薬剤が光ハプテンとしての性格を持つため，紫外線照射により表皮ランゲルハンス細胞に共有結合し，T細胞を感作・惹起することにより発症する．

はじめに

光線過敏症は日光などの照射を受けた皮膚に生じる皮膚炎の総称であり，種々多様な原因で起こる．内服薬剤によるものは成人の光線過敏症患者を診た場合，まず最初に考えるべきである．この薬剤性光線過敏症は一方では，薬疹という分類の観点からもとらえることができる．すなわち皮疹が起こるために光照射を必要とするタイプの薬疹があり，これを光線過敏型薬疹と呼ぶ．したがって，薬剤性光線過敏症は光線過敏症から見た分類，光線過敏型薬疹は薬疹から見た分類であり，両者は同義語である．

臨床症状

通常，薬剤内服中に戸外で日光に曝露されたというエピソードがあって発症する．老人ではいつ日光に曝されたかはっきりしないことも多く，また病室の窓際にベッドが位置していたために起こることもある．皮疹の分布に特徴があり，顔面，口唇とくに下口唇，耳介，項部，上胸部V領域，手背(図1)などの露光部位に限局して皮疹がみられる．半袖，半ズボンで日光に曝露された時には，前腕伸側，下肢伸側にも皮膚炎は生じ，またサンダル，下駄履きの場合には，足背にも皮疹が生ずる．

光毒性反応は日焼け(サンバーン)様発疹をとり，光アレルギー性の場合は，浮腫性紅斑，水疱，扁平苔癬様皮疹，白斑黒皮症などさまざまである．時には光毒性反応を思わす浮腫性紅斑で始まり，経過とともに扁平苔癬様皮疹に変化する症例もある．このことは同一患者内，同一エピソード内でも光毒性反応と光アレルギー性反応が連続的に起こりうることを推察させる．扁平苔癬様皮疹の性状は，紅斑ではあるが色が紫がかっていることにある．急性反応的でないため，しばしば光線過敏症を思い浮かべることが難しい．色素沈着と色素脱失が混在する病変は，白斑黒皮症と称される(図2)．原因である薬剤内服を中止することが遅れ，長期に光線過敏性皮膚炎を患った患者に多い．すでに完成してしまっている状態では難治である．

* Yoshiki TOKURA，〒807-8555 北九州市八幡西区医生ヶ丘1-1 産業医科大学皮膚科学教室，教授