Complement-derived Chemotactic Activity
Is Generated in Human Serum Containing
Uroporphyrin after Irradiation with 405 nm Light

HENRY W. LIM, H. DANIEL PEREZ, IRA M. GOLDSCHMIDT, and IRMA GIGLI,
Departments of Dermatology and Medicine, New York University Medical
Center, New York 10016

ABSTRACT Patients with porphyrias have varying degrees of photosensitivity, associated with elevated levels of porphyrins in plasma, erythrocyte, urine and/or feces. To investigate the role of complement in the pathogenesis of cutaneous lesions, varying amounts of uroporphyrin were added to normal human serum (0.1–10 µg/ml), and the mixtures were then exposed to 405 nm irradiation. Such treatments result in the diminution of total hemolytic complement activity and hemolytic titers of C1, C4, C2, C3, and C5; furthermore, cleavage products of C3 and C5 were detected. Chemotactic activity for human polymorphonuclear leukocytes was generated that was inhibitable by incubation with anti-C5, but not with anti-C3 antisera. No chemotactic activity was generated in Mg++-EGTA treated serum nor in C4-deficient guinea pig serum. These data indicate that irradiation with 405 nm light of normal human serum containing uroporphyrin results in activation of the complement system via the classical pathway, and the generation of complement (C5)-derived chemotactic activity for human polymorphonuclear leukocytes.

INTRODUCTION

Porphyrias are a group of metabolic diseases characterized by elevated levels of porphyrins in plasma, erythrocytes, urine, and/or feces. Upon exposure to sunlight, most patients develop edema and erythema of the skin, with subsequent formation of blisters. Scarring occurs after repeated attacks (1).

Exposure of patients with erythropoietic protoporphyruria to light containing the action spectrum of porphyrin molecules (405 nm) results in lysis of capillary endothelial cells, mast cell degranulation, and the appearance of polymorphonuclear leukocytes (PMN)1 in the dermis (2). Direct immunofluorescence studies of exposed skin from patients with porphyrias demonstrated deposition of C3 and immunoglobulins around blood vessels and at the dermo-epidermal junction (3, 4). These observations, together with our finding of decreased levels of complement in serum from patients with erythropoietic protoporphyrinia after irradiation in vitro (5), prompted us to consider the possibility that products of complement activation may play a role in the pathogenesis of cutaneous lesions in the porphyrias. Recently, using an animal model, we have obtained evidence in support of this possibility. We were able to demonstrate that in guinea pigs injected intraperitoneally with porphyrins, irradiation with 405 nm light produced cutaneous lesions as well as marked diminution of total serum hemolytic complement activity (6).

We now present data indicating that irradiation with 405 nm light of normal human serum containing uroporphyrin results in activation of the complement system via the classical pathway and generation of complement (C5)-derived chemotactic activity for human PMN.

METHODS

Source of serum. Pooled sera from healthy adult volunteers were processed and used as previously described (5). Serum from a patient with hereditary deficiency of C5 was generously provided by Dr. Ralph Synderman. C4-deficient

1 Abbreviation used in this paper: PMN, polymorphonuclear leukocytes.
guinea pig serum was obtained from our C4-deficient guinea pig colony at New York University Medical Center.

**Light source.** Four General Electric F40BL tubes (General Electric Co., Medical Systems Div., Milwaukee, Wis.) were used as the source of 405 nm light. Photons emitted over a wavelength range of 320–450 nm represent 94% of the total energy output of the lamps. Using an IL 700 research radiometer (International Light, Inc. Newburyport, Mass.) and a 3-mm window-glass as a filter, the output of the lamps at 15 cm was as follows: 275–303 nm, 2.13 μW/cm² (SEE 240 sensor, UVB 63 filter, International Light, Inc.); 308–318 nm, 13.4 μW/cm² (PT171C sensor, NB 313 filter); 330–390 nm, 1,310 μW/cm² (PT171C sensor, WB 365 filter); and 400–410 nm, 200 μW/cm² (PT171C sensor, NB 405 filter).

The ultraviolet-B light source was a barrel of four Westinghouse FS40BL tubes (Westinghouse Electric Corp., Bloomfield, N. J.), which emit photons over the wavelength range of 275–375 nm. The output of the lamps at 15 cm was as follows: 275–303 nm, 307 μW/cm² (SEE 240 sensor, UVB 63 filter); 308–318 nm, 495 μW/cm² (PT171C sensor, NB 313 filter); 330–390 nm, 680 μW/cm² (PT171C sensor, WB 365 filter); and 400–410 nm, 44 μW/cm² (PT171C sensor, NB 405 filter).

**Irradiation of serum.** Uroporphyrin I dihydrochloride (Porphyrin Products, Logan, Utah) was dissolved in phosphate (10 mM)-buffered 140 mM NaCl, pH 7.4, and added to normal human sera to yield final desired concentrations. To eliminate the effect of ambient ultraviolet irradiation, all procedures involving uroporphyrin were carried out under red incandescent light. The mixtures were then irradiated on ice with 405 nm light, as previously described (6). To evaluate the effect of radiation of less than 320 nm emitted from the 405 nm light source used, serum containing uroporphyrin (5.0 μg/ml) was exposed to 24.8 ml/cm² of 308–318 nm irradiation from the ultraviolet-B light source.

**Hemolytic titration of total complement activity and individual complement components.** Buffers and cellular intermediates used for complement hemolytic titration (veronal-buffered saline containing gelatin [GVB]), GVB containing CaCl₂ and MgCl₂ [GVB**], GVB containing dextrose [DGVB**], EA, EAC1, and EACT4) were prepared as previously described (7). These were used to measure hemolytic titers of total complement activity, C1, C4, C2, C3, and C5 (6, 7).

**Crossed immunoelectrophoresis.** Two-dimensional (crossed) immunoelectrophoresis was performed employing minor modifications of the method of Laurell (8). Goat antisera to human C3 and human C5 were obtained from Meloy Laboratories, Inc., Springfield, Va.

**PMN chemotaxis.** PMN were obtained from venous blood of a healthy adult donor by a previously described method (9). PMN random motility and directed migration (chemotaxis) were assessed by using a minor modification of the “leading front” method of Zieg mond and Hirsch (8). Aliquots (0.8 ml) of leukocyte suspensions (containing 2.5 × 10⁷ PMN/ml) were added to the upper compartments of modified Boyden chambers (Nucleopore Corp., Pleasanton, Calif.) Dilutions of treated and untreated sera, or buffer, were placed in the lower compartments. Chambers containing cells and chemotactic stimuli were incubated at 37°C for 35 min in an atmosphere of 5% CO₂ and 100% humidity. The filters were then removed, fixed in methanol, stained with hematoxylin, dehydrated in ethanol, and cleared in xylene. The response of PMN either to buffer alone (random motility) or to chemotactic stimuli is reported as the distance that the leading front of cells migrated into the filters (micrometers/35 min).

**RESULTS**

Effect of 405 nm irradiation on the activity of complement in normal human serum containing uroporphyrin. Irradiation for 30 min of normal human serum containing uroporphyrin (0.6–10 μg/ml) resulted in a marked diminution of total hemolytic complement activity, hemolytic titers of C3 and C5 (Fig. 1), as well as titers of C1, C4, and C2 (not shown). Changes in complement hemolytic activity were dependent upon the concentration of uroporphyrin in serum. Significant changes were observed at a uroporphyrin concentration of 0.6 μg/ml. No alteration of hemolytic complement activity was observed in uroporphyrin-containing serum that was kept in the dark, nor in irradiated normal human sera that did not contain uroporphyrin. The diminution of complement activity was associated with the generation of cleavage products of C3 and C5, as evidenced by the appearance of an additional anodal precipitant peak on two-dimensional immunoelectrophoresis when uroporphyrin-containing serum was irradiated. Irradiation of uroporphyrin-containing serum with ultraviolet-B did not result in any alteration of complement hemolytic activity (data not shown). Furthermore, the effect of 405 nm irradiation on complement activity was dependent on the duration of irradiation (data not shown).

<table>
<thead>
<tr>
<th>Residual Hemolytic Activity (%)</th>
<th>Uroporphyrin Concentration (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>CH50</td>
</tr>
<tr>
<td>80</td>
<td>C3</td>
</tr>
<tr>
<td>60</td>
<td>C5</td>
</tr>
<tr>
<td>40</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td></td>
</tr>
<tr>
<td>0</td>
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</table>

**FIGURE 1** Effect of serum concentration of uroporphyrin on total hemolytic complement (CH50), C3, and C5 activities.

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TABLE I

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Total migration</th>
<th>Net migration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer (random migration)</td>
<td>95.2±1.9</td>
<td>—</td>
</tr>
<tr>
<td>NHS + URO (5 µg/ml) (nonirradiated)</td>
<td>96.8±1.7</td>
<td>1.6±2.6</td>
</tr>
<tr>
<td>NHS + URO (5 µg/ml) + irradiation§</td>
<td>115.0±0.8µ</td>
<td>19.8±2.1</td>
</tr>
<tr>
<td>NHS + URO (5 µg/ml) + irradiation → 56°C × 30 min</td>
<td>114.1±0.6µ</td>
<td>18.9±2.0</td>
</tr>
<tr>
<td>NHS + URO (5 µg/ml) + irradiation → anti-human C5</td>
<td>93.5±1.1</td>
<td>0±2.2</td>
</tr>
<tr>
<td>NHS + URO (5 µg/ml) + irradiation → anti-human C3</td>
<td>113.9±2.0µ</td>
<td>18.7±2.8</td>
</tr>
<tr>
<td>Zymosan-activated serum</td>
<td>123.5±2.5µ</td>
<td>28.3±3.1</td>
</tr>
</tbody>
</table>

* Results represent the mean of three experiments (±SEM). Triplicate chambers were used in each experiment and 10 fields were examined in each filter.
§ Directed motility minus random migration.
§§ Normal human serum (NHS) containing uroporphyrin (URO) was irradiated with 405 nm light for 30 min at 4°C (as described in Methods).

Activity in normal human serum was dependent upon the concentration of uroporphyrin (Fig. 2), with 10 µg/ml of uroporphyrin generated chemotactic activity of the same magnitude as that generated in zymosan-activated serum. Furthermore, the chemotactic activity generated in serum containing 0.1 µg/ml of uroporphyrin was dependent upon the duration of the irradiation (Fig. 3).

The chemotactic activity was characterized further by molecular sieve chromatography. 1 ml of irradiated human serum containing uroporphyrin (5.0 µg/ml) was applied to a 2.5 × 40-cm calibrated column of Sephadex G-75 (Pharmacia Fine Chemicals, Piscataway, N. J.).

Elution was performed with phosphate (10 mM)-buffered 140 mM NaCl, pH 7.4, at a flow rate of 10 ml/h. 3 ml fractions were collected and assayed for chemotactic activity. As shown in Fig. 4, a single peak of chemotactic activity was eluted with apparent molecular weight of 15,000. The chemotactic activity in these fractions was heat-stable (56°C for 30 min), and inhabitable by incubation with anti-human C5 but not with anti-human C3 antisera. This elution profile was similar to the elution profile of C5-derived chemotactic activity observed when zymosan-treated serum was chromatographed under identical conditions (8).

These data indicate that the chemotactic activity generated by irradiation of serum containing uroporphyrin is derived primarily from the fifth component of

![Figure 2](image1.png)

**Figure 2** Effect of serum concentration of uroporphyrin on the generation of chemotactic activity after 30 min of irradiation. Results are expressed as net chemotaxis (directed motility minus random migration).

![Figure 3](image2.png)

**Figure 3** Effect of duration on chemotactic activity in serum containing 0.1 µg/ml of uroporphyrin. Results are expressed as net chemotaxis.
complement (C5). This was confirmed in experiments using serum from a patient with hereditary deficiency of C5 (Table II). C5-deficient serum containing uroporphyrin did not yield any chemotactic activity after exposure to 405 nm light; upon reconstitution of the C5-deficient serum with purified human C5, chemotactic activity was generated.

**Participation of the classical pathway of complement activation in the generation of chemotactic activity.** To determine which pathway of complement activation is involved in the generation of chemotactic activity, uroporphyrin was added to human serum that had been rendered 2.0 mM with respect to MgCl₂ and 20 mM with respect to EGTA, to effectively block activation of the classical complement pathway (10). As shown in Table II, when such serum was irradiated, it failed to yield chemotactic activity. To further demonstrate the participation of the classical pathway, C4-deficient guinea pig serum was used. Irradiation of normal guinea pig serum containing uroporphyrin resulted in the generation of significant chemotactic activity, whereas no chemotactic activity could be detected when C4-deficient guinea pig serum was treated under identical conditions. These results indicate that an intact classical pathway of complement activation is required for the generation of chemotactic activity in irradiated serum containing uroporphyrin.

**DISCUSSION**

The results of the experiments described in this report indicate that irradiation of normal human serum containing uroporphyrin with light having the action spectrum of porphyrin molecules (405 nm) results in activation of the complement system via the classical

**TABLE II**

Evidence for the Requirement of Native C5 and an Intact Classical Complement Pathway for the Generation of Chemotactic Activity by 405 nm Irradiation of Serum Containing Uroporphyrin

<table>
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<tr>
<th>Stimulus</th>
<th>Total migration</th>
<th>Net migration</th>
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<tbody>
<tr>
<td>Buffer (random migration)</td>
<td>96.5±2.0</td>
<td>—</td>
</tr>
<tr>
<td>NHS + URO (5 µg/ml) + irradiation §</td>
<td>121.6±2.2</td>
<td>25.1±3.0</td>
</tr>
<tr>
<td>C5-deficient human serum + URO (5 µg/ml) + irradiation §</td>
<td>94.3±1.7</td>
<td>0±2.6</td>
</tr>
<tr>
<td>C5-deficient human serum + C5 + URO + irradiation</td>
<td>114.4±2.2</td>
<td>11.9±3.0</td>
</tr>
<tr>
<td>NHS + URO (5 µg/ml) + Mg²⁺-EGTA + irradiation</td>
<td>101.0±2.7</td>
<td>4.5±3.4</td>
</tr>
<tr>
<td>GPS + URO (5 µg/ml) + irradiation §</td>
<td>99.7±2.9</td>
<td>3.2±3.5</td>
</tr>
</tbody>
</table>

* Results represent the mean of three experiments (±SEM). Triplicate chambers were used in each experiment and 10 fields were examined in each filter.

† Directed motility minus random migration.

§ Normal human serum (NHS), guinea pig serum (GPS), and complement-deficient sera containing uroporphyrin (URO) were irradiated with 405 nm light for 30 min at 4°C (as described in Methods).

* Sample vs. control < 0.01 (Student's t test).
pathway and generation of complement (C5)-derived chemotactic activity for human PMN. Decreases in hemolytic titers of total hemolytic complement, C3, C5 (Fig. 1), C1, C4, and C2 were dependent upon the concentration of uroporphyrin added to the serum and on the duration of irradiation. That the specific action spectrum of porphyrins was required for this effect was demonstrated by the lack of any alteration of complement profile in serum containing uroporphyrin that was exposed to ultraviolet-B irradiation.

Generation of chemotactic activity for human PMN also was dependent upon the concentration in serum of uroporphyrin and on the duration of irradiation (Table I, Figs. 2 and 3). The chemotactic activity resisted heating at 56°C for 30 min and it was inhibited completely by treatment with antisera to human C5 (Table 1); it could be eluted from Sephadex G-75 column in a single peak with an apparent molecular weight of 15,000 (Fig. 4). This elution profile was identical to the elution profile of C5-derived chemotactic activity observed after chromatography of serum that had been activated with zymosan. Furthermore, serum from a patient with hereditary deficiency of C5 failed to yield chemotactic activity unless it was reconstituted with purified human C5 (Table II). These results indicated that the chemotactic activity generated is derived from C5.

Evidence that the classical pathway of complement activation is essential for the generation of chemotactic activity in irradiated serum containing uroporphyrin was provided by the experiments summarized in Table II. Neither human serum treated with Mg2+-EGTA (which blocks activation of the classical complement pathway) (10) nor C4-deficient guinea pig serum yielded chemotactic activity. The mechanism by which the classical pathway is activated however, remains to be elucidated.

Solar irradiance (400-410 nm) at sea level with the sun directly overhead is ~1,100 μW/cm² (11). This is more than five times greater than the output of the light source used in these experiments (200 μW/cm² measured at 15 cm). Taking into account that the total transmission of light in the wavelength range of 400-410 nm through the epidermis of Caucasians is ~50% (12), it is clear that a similar dose of radiation energy as that used in these experiments could be attained in the dermis by exposure to sunlight in the course of regular daily activities. Furthermore, significant chemotactic activity was generated in serum containing 0.1 μg/ml of uroporphyrin (Fig. 3), a concentration similar to that observed in serum from patients with porphyria cutanea tarda (13).

The pathogenesis of cutaneous lesions in the porphyrias is poorly understood. Lysis of endothelial cell following 405 nm irradiation has been observed in animal models (14) and in patients (2); this has been attributed to the action of oxygen-derived free radicals and peroxides (15). The following observations suggest that complement also may play an important role: deposition of complement components in exposed skin of patients with porphyria cutanea tarda (3), porphyria variegata (3), and erythropoietic protoporphyria (3, 4); loss of hemolytic complement activity in sera from patients with erythropoietic protoporphyria upon 405 nm irradiation (5) and in sera from guinea pigs that had been injected intraperitoneally with porphyrins (6). Histologically, in patients as well as in animals, there is dermal edema immediately after irradiation, followed by infiltration with PMN and degranulation of mast cells (2, 6, 14). In this report, we have presented data indicating that complement (C5)-derived chemotactic activity is generated by 405 nm irradiation of serum containing uroporphyrin. These data, taken together with our recent observations that irradiation of sera from patients with erythropoietic protoporphyria results in the generation of chemotactic activity for human PMN (16), suggest the possibility that complement (C5)-derived peptides play an important role in the pathogenesis of cutaneous lesions in the porphyrias. Such peptides, generated by 405 nm irradiation in the papillary dermis, can increase vascular permeability, resulting in dermal edema, and provoke local accumulation of PMN (17), resulting in further tissue damage and amplification of the inflammatory process. Thus, complement-mediated inflammation and tissue injury may account, at least in part, for the cutaneous lesions that develop in patients with porphyria.

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