Activated Complement Component 3 (C3) Is Required for Ultraviolet Induction of Immunosuppression and Antigenic Tolerance

By Craig Hammerberg,* Santosh K. Katiyar,* Michael C. Carroll,‡ and Kevin D. Cooper*§

From the *Department of Dermatology, Case Western Reserve University, and University Hospitals of Cleveland, Cleveland, Ohio 44106; the ‡Department of Pathology, Harvard Medical School, Boston, Massachusetts 02115; and the §Veterans Affairs Hospital, Cleveland, Ohio 44106

Summary

Complement component 3 (C3), a critical regulator of innate immunity, may also play a role in the regulation of cognate immunity, such as contact sensitivity responses. Because ultraviolet (UV) radiation also activates C3 in the skin, we determined whether the immunosuppressed state that results when a contact sensitizer is applied through UVB-exposed skin requires the presence and activation of C3. This question was addressed through the use of C3-deficient mice, blockade of C3 cleavage to C3b, and accelerated degradation of iC3b by soluble complement receptor 1 (sCR1). Both C3-modulated systems totally reversed the failure to induce a contact sensitivity response to dinitrofluorobenzene (DNFB) upon primary sensitization at the UV-exposed site, as well as immunologic tolerance to a second DNFB immunization through normal skin. Treatment with sCR1 reduced the infiltration of CD11b+ leukocytes into the epidermis and dermis of UV-irradiated skin but did not reverse the UV-induced depletion of epidermal class II MHC+CD11blo Langerhans cells. These data, taken together with previous results showing abrogation of locally induced UV immunosuppression by in vivo anti-CD11b treatment, suggest a novel mechanism by which ligation of the leukocyte b2 integrin, CD11b, by iC3b molecules formed from C3 activation in UV-exposed skin, modifies cutaneous CD11b+ cells such that skin antigen-presenting cells are unable to sensitize in a primary immune response, but actively induce antigenic tolerance.
Materials and Methods

Mice. C3H/HeN females were purchased from Charles River (Wilmington, MA). (129 × C57BL/6)F1 females were purchased from the Jackson Laboratory (Bar Harbor, ME). C3-deficient females were bred at the Harvard Medical School (Boston, MA). All mice were between the ages of 6 and 12 weeks of age. Groups of four mice were used for each panel of the contact sensitivity experiments.

Antibodies and Reagents. 2,4-dinitro-1-fluorobenzene (DNFB) was purchased from Sigma Chemical Co. (St. Louis, MO). Soluble CR1 was a gift from T Cell Sciences, Inc. (Needham, MA). Ethidium monoazide and Cascade blue-conjugated avidin were obtained from Molecular Probes (Eugene, OR). PE-conjugated rat anti-CD11b (rat IgG2b, clone M1/70) was purchased from Boehringer Mannheim (Indianapolis, IN). Biotin-conjugated mouse anti-Ia (mouse IgG2b, clone 11-5.2) and FITC-conjugated rat anti-mouse Gr-1 (rat IgG2b, clone R86-8C5) were obtained from PharMingen (San Diego, CA).

UV Induction of Tolerance. Razor-shaved and chemically depilated mouse skin was exposed as previously described (15) to UVB irradiation from a band of six FS-40 fluorescent lamps from which UV and UVB wavelengths were not present in natural solar radiation. The UVB emission was measured with an IL-443 photosensitivity radiometer (International Light, Newburyport, MA) equipped with an IL SED 240 detector fitted with a Wide angle quartz diffuser and a SC5 280 filter. A single UV exposure was administered to each strain at a dose such that tolerance was induced only if DNFB was applied to the UV-exposed skin and not a non–UV-exposed distant site (low-dose, locally inducible tolerance). The UVB dose was determined (data not shown) that induced tolerance to DNFB; UV-exposed C3-deficient mice showed no discernible effect of UV upon contact sensitivity induction in a C3-deficient environment. As done previously (17), a single UVB dose was determined (data not shown) that induced tolerance only when the DNFB was applied 48 h after UV irradiation to the UV-exposed skin (locally induced tolerance) and not at a distant non–UV-exposed site (systemically induced tolerance). Based upon these criteria, a single UVB dose of 140 mJ/cm² for the C3-deficient mice and their (129 × C57BL/6)F1 controls was used. The absence of C3 did not affect the ability of C3-deficient mice to mount a contact sensitivity response to DNFB (Fig. 1, bar 2 from the top) that was comparable to that observed in (129 × C57BL/6)F1 controls (Fig. 1, bar 5). By contrast, C3-deficient mice differed markedly from their (129 × C57BL/6)F1 controls; absence of C3 conferred protection from UV's ability to inhibit contact sensitization (Fig. 1, left, bar 6 [UV inhibition] versus 5 [no UV sensitization in C3 sufficient controls]), as compared to no reduction in sensitization after UV in C3-deficient mice (bar 3 versus 2). Similarly, C3-deficient mice were protected against the development of tolerance to DNFB; UV-exposed C3-deficient mice were not blocked in their ability to be resensitized through normal skin (Fig. 1, right, bars 2 and 3), as were normal monoazide (15). Stained cells were fixed in 1% paraformaldehyde before flow cytometric analysis by an Epics Elite Cytoflex (Coulter Electronics, Hialeah, FL). Positive cell percentages were determined by calculating the percentage of the positive cell population in terms of total viable epidermal or dermal cells and subtracting the percentage of cells found within the same positive gate in the isotype control–stained samples.

Results

A absence of C3 protected UV-induced tolerance to a contact sensitizer. Mice deficient in C3 due to the disruption of the C3 gene, which resulted in a mouse strain with undetectable levels of serum C3 (13), were assayed for the effect of UV upon contact sensitivity induction in a C3-deficient environment. As done previously (17), a single UVB dose was determined (data not shown) that induced tolerance only when the DNFB was applied 48 h after UV irradiation to the UV-exposed skin (locally induced tolerance) and not at a distant non–UV-exposed site (systemically induced tolerance). Based upon these criteria, a single UVB dose of 140 mJ/cm² for the C3-deficient mice and their (129 × C57BL/6)F1 controls was used. The absence of C3 did not affect the ability of C3-deficient mice to mount a contact sensitivity response to DNFB (Fig. 1, bar 2 from the top) that was comparable to that observed in (129 × C57BL/6)F1 controls (Fig. 1, bar 5). By contrast, C3-deficient mice differed markedly from their (129 × C57BL/6)F1 controls; absence of C3 conferred protection from UV's ability to inhibit contact sensitization (Fig. 1, left, bar 6 [UV inhibition] versus 5 [no UV sensitization in C3 sufficient controls]), as compared to no reduction in sensitization after UV in C3-deficient mice (bar 3 versus 2). Similarly, C3-deficient mice were protected against the development of tolerance to DNFB; UV-exposed C3-deficient mice were not blocked in their ability to be resensitized through normal skin (Fig. 1, right, bars 2 and 3), as were normal monoazide (15). Stained cells were fixed in 1% paraformaldehyde before flow cytometric analysis by an Epics Elite Cytoflex (Coulter Electronics, Hialeah, FL). Positive cell percentages were determined by calculating the percentage of the positive cell population in terms of total viable epidermal or dermal cells and subtracting the percentage of cells found within the same positive gate in the isotype control–stained samples.

![Figure 1](image-url)
mice (Fig. 1, right, bars 5 and 6). Thus, although C3 is not needed for positive immune response development in this model, C3 does appear to be critical for UV-induced downregulatory responses.

C3 Activation Required for UV Induction of Immunosuppression. We next determined whether C3 activation was required for the locally induced effects of UV upon suppression of contact sensitivity responses. C3 activation can be blocked in vivo by sCR1, which both inactivates C3 convertase (and C5 convertase) and accelerates degradation of iC3b (14, 18) and in the mouse inhibits the alternative pathway of C3 activation (19). Relative to non-sCR1–treated C3H/HeN mice (Fig. 2, bar 2 from top), sCR1 treatment did not affect the contact sensitivity response to DNFB after either the primary or secondary challenge (Fig. 2, bar 3). We have previously demonstrated that for C3H/HeN, 72 mj/cm² is sufficient to create the local skin milieu that can induce tolerance, but is not high enough to also generate systemically acting factors that can induce tolerance even upon immunization through normal skin (17). UV irradiation with 72 mj/cm² did inhibit both the primary and secondary contact sensitivity response in non-sCR1–treated mice (Fig. 2, bar 4). However, sCR1 treatment of UV-exposed C3H/HeN mice completely prevented UV injury of the skin’s ability to induce a primary contact sensitivity response (Fig. 2, left, bar 5). Furthermore, sCR1 fully reversed UV induction of tolerance (active suppression of ability to sensitize) (Fig. 2, right, bar 5).

Effect of sCR1 Treatment on UV Modulation of Skin Leukocyte Populations. We have previously observed that treating mice with blocking antibodies to CD11b, the receptor for iC3b, blocked CD11b⁺ leukocyte infiltration into both the dermis and epidermis of UV-exposed skin (12). Since sCR1 treatments block the UV-induced formation or stable accumulation of the CD11b ligand iC3b (data not shown), it was determined whether sCR1 treatment also affects leukocyte movement into UV exposed skin. Because skin-infiltrating leukocytes express both CD11b and Gr-1 (17), and the tolerogenic cells in UV-exposed epidermis express CD11b⁺Gr-1⁺ LC, three-color flow cytometric analysis was used to determine the expression of CD11b and Gr-1 by epidermal cells obtained from the skin of C3H/HeN mice (A and B), 48 h after UV exposure, that had been treated with either PBS (A) or sCR1 (B). Cells were first selected based upon their viability (EMA exclusion), then analyzed for CD11b expression (x-axis represents PE intensity) and Gr-1 expression (y-axis represents FITC intensity). Cells stained with the matching antibody isotype were confined to quadrant 3. Percentage of stained cells after the subtracting the isotype background are given for each quadrant. Four-color flow cytometric analysis was used to identify class II MHC⁺CD11b⁺Gr-1⁺ LC from UV-exposed epidermis of C3H/HeN mice with (D) or without (C) sCR1 treatment. Determination of the expression of CD11b (y-axis represents PE intensity) and Gr-1 (x-axis represents FITC intensity) on class II MHC⁺ epidermal cells electronically selected by their reactivity to biotin-conjugated anti-Iaα and avidin-Cascade blue was determined by flow cytometric analysis. For the preparation of epidermal cell suspensions six to eight mice were used in each group.

Figure 2. Reversal by sCR1 of induction of tolerance to a contact sensitizer immunized through skin of C3H/HeN mice exposed to a single UVB dose (72 mj/cm²). The contact sensitivity response to DNFB of mice receiving no UV with and without sCR1 treatment is given in the third and second bars from the top, respectively. The response of mice receiving UV with no sCR1 is shown in the fourth bar from the top, and with sCR1 in the fifth bar from the top. The left panel shows the ear swelling response to primary sensitization through dorsal skin and challenge. The right panel indicates the response to secondary sensitization through normal back skin and rechallenge. Data are expressed as described in Fig. 1. Four mice were used in each group.

Figure 3. sCR1 treatment of UV-irradiated C3H/HeN mice partially blocks infiltration of CD11b⁺ leukocytes but does not prevent UV-induced depletion of class II MHC⁺CD11b⁺Gr-1⁺ LC. Three-color flow cytometric analysis was used to determine the expression of CD11b and Gr-1 by epidermal cells obtained from the skin of C3H/HeN mice (A and B), 48 h after UV exposure, that had been treated with either PBS (A) or sCR1 (B). Cells were first selected based upon their viability (EMA exclusion), then analyzed for CD11b expression (x-axis represents PE intensity) and Gr-1 expression (y-axis represents FITC intensity). Cells stained with the matching antibody isotype were confined to quadrant 3. Percentage of stained cells after subtracting the isotype background are given for each quadrant. Four-color flow cytometric analysis was used to identify class II MHC⁺CD11b⁺Gr-1⁺ LC from UV-exposed epidermis of C3H/HeN mice with (D) or without (C) sCR1 treatment. Determination of the expression of CD11b (y-axis represents PE intensity) and Gr-1 (x-axis represents FITC intensity) on class II MHC⁺ epidermal cells electronically selected by their reactivity to biotin-conjugated anti-Iaα and avidin-Cascade blue was determined by flow cytometric analysis. For the preparation of epidermal cell suspensions six to eight mice were used in each group.
CD11b by monocytes/macrophages (17). Normal levels of class II MHC-CD11b+ LC are ~4% (17); as a result of UV irradiation, this population drops to 0.2% in C3H/HeN (Fig. 3 C) mice. However, sCR1 pretreatment of UV-irradiated C3H/HeN mice did not affect the UV-induced class II MHC-CD11b+ LC depletion (Fig. 3 D, no restoration of LC in quadrant F). Shown is the experiment that resulted in the median value from three separate experiments of six to eight mice in each group. Similar results were observed by staining for LC with anti-class II MHC antibodies in tissue sections of skin from UV-irradiated and sCR1-pretreated plus UV-irradiated C3H/HeN mice (data not shown).

Discussion

Using two different in vivo mouse model systems in which either C3 is deficient (C3 gene disruption) or C3 is present and activation of C3 is inhibited (sCR1), it was demonstrated that C3 is essential for UV induction of an immunosuppressed state to a contact sensitizer. The UV dose used for each mouse system was such that only when the contact sensitizer was applied to the UV-irradiated skin, and not at a distant site, was tolerance induced. Using such UV doses in both C3 mouse models, reversal of UV induction of an immunosuppressed state was complete, indicating not only that C3 is required but that activation of C3 (sCR1 model) is essential. Treatment of normal mice with sCR1 did not alter their ability to generate a contact sensitivity response, indicating that sCR1 is not affecting the normal antigen-presenting cell–T cell interaction that results in contact sensitivity. Because the UV dose used was local in its effect on contact sensitization induction, this would imply that the influence of UV-induced C3 activation upon the generation of a contact sensitization response is also restricted to the skin at the site of UV irradiation.

The complement components required for the activation of C3 by the alternative pathway are all present in the skin. C3 and its activating protease, factor B, have both been detected in normal epidermis (6). In addition, production of C3 and factor B by cultured keratinocytes has been demonstrated (6–8). Cleavage fragments of C3 have also been observed in the normal epidermal basement membrane zone (21, 22). Moreover, complement deposits of C3b and C3d have been detected in and on epidermal cells within 24 h of exposure of the skin to UVB, indicating activation of C3 by UVB (10). Treatment with sCR1 eliminated the presence of C3 deposits (data not shown), indicating that the activation of C3 in the skin by UV was blocked by sCR1 (inhibiting C3 cleavage to C3b).

sCR1 treatment of UV-irradiated C3H/HeN mice resulted in a decreased, but not total inhibition of, leukocyte infiltration into the UV-irradiated skin and little restoration of class II MHC+ cells. Specifically, a recovery of class II MHC-CD11b+ LC was not observed in sCR1-treated, UV-exposed C3H/HeN mice where sCR1 treatment also restored a primary contact sensitivity response as well as blocked tolerance induction. The lack of retention of LC after UV when C3 degradation is blocked indicates the effect is not due to iC3b triggering of tolerogenic UV–LC emigration. This finding implicates the involvement of products of UV-induced C3 activation in the stimulation of the development of a tolerance-inducing antigen-presenting cell. C3a, a product of the initial cleavage of C3, is capable of activating leukocytes as measured by increased reactive oxygen species production (23). More direct evidence for the involvement of C3 fragments in modifying the development of antigen-presenting cells derives from findings that C3b, the second fragment resulting from the initial cleavage of C3, cross-links the C3b receptor (CD46), resulting in inhibition of induction of IL-12 production (24). IL-12 is critical for the successful induction of contact sensitivity and reverses UV-induced immune suppression (25–28). We have found that a deficiency of IL-12-producing cells within the draining lymph nodes of UV-exposed, contact-sensitized mice is associated with the induction of tolerance in these animals (29).

However, C3b is highly unstable and rapidly undergoes further cleavage by factor I, in the presence of factor H, resulting in the generation of the more stable product, iC3b, that binds to its receptor, CR3 (CD11b/CD18). Stimulation of leukocytes through C3dg over iC3b results in activation of leukocytes (30). Cross-linking of C3dg and CD11b integrin on leukocytes results in increased tyrosine phosphorylation (31, 32), changes in intracellular calcium levels (33–35), and increased reactive oxygen species production (33, 34, 36). Thus, C3 fragments generated in UV-irradiated skin may, through binding to their receptors, be providing a critical signal necessary for the development of an antigen-presenting cell capable of inducing T suppressor cells. Our previous data demonstrating complete blockade of UV-induced tolerance by in vivo anti-CD11b treatment and only partial blockade of CD11b+ leukocyte infiltration (12) suggests the additional involvement of the CD11b molecule (iC3b receptor) in tolerance induction. This hypothesis is supported by recent publications that demonstrate cross-linking the CD11b/CD18 molecule downregulates induction of interleukin-12 by monocytes (37, 38). Furthermore, we have recently found that iC3b is capable of inducing IL-10 in human monocytes (39).

Therefore, in our model both infiltrating and indigenous CD11b-expressing antigen-presenting cells would be triggered by UV-activated C3 into a state of differentiation/activation that is insufficient to support initiation of a primary immune response, but in fact induces antigenic tolerance. However, in the absence of the iC3b/CD11b signal, the infiltrating and indigenous (at least those remaining after UV) antigen-presenting cells would acquire functional properties (IL-12, costimulatory molecules) that support initiation of a primary immune response. These results implicate a novel mechanism of immunoregulation that can be operative or manipulated in UV injury, UV tumor immunity, photosensitivity, and other cutaneous immune-mediated diseases.
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Address correspondence to Kevin D. Cooper, Department of Dermatology, Case Western Reserve University Hospitals of Cleveland, 11000 Euclid Ave., Cleveland, OH 44106. Phone: 216-368-0533; Fax: 216-368-0212.

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