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Mast Cell-Derived IL-10 Suppresses Germinal Center Formation by Affecting T Follicular Helper Cell Function

Rommel Chacón-Salinas,*,†,‡,§ Alberto Y. Limón-Flores, *,†,‡,§ Alma D. Chávez-Blanco, *,§ Alexei Gonzalez-Estrada,*,∥ and Stephen E. Ullrich*,∥


The most prevalent cancer diagnosed in the world is sunlight-induced skin cancer. In addition to being a complete carcinogen, UV radiation, the causative agent of skin cancer, induces immune suppression. Because UV-induced immune suppression is a well-recognized risk factor for skin cancer induction, it is crucial to understand the mechanisms underlying UV-induced immune suppression. Mast cells, which have recently emerged as immune regulatory cells, are particularly important in UV-induced immune suppression. UV exposure does not induce immune suppression in mast cell-deficient mice. We report that UV irradiation blocks germinal center (GC) formation, Ab secretion, and T follicular helper (Tfh) cell function, in part by altering the expression of transcription factors BCL-6 and BLIMP-1. No suppression of GC formation, Tfh cell IL-21 expression, or Ab secretion was observed in UV-irradiated mast cell-deficient (KitW-sh/W-sh) mice. When mast cell-deficient mice were reconstituted with wild type mast cells, immune suppression was restored. Reconstituting the mast cell-deficient mice with bone marrow-derived mast cells from IL-10-deficient mice failed to restore the ability of UV radiation to suppress GC formation. Our findings demonstrate a function for mast cells, suppression of Tfh cell production, GC formation, and Ab production in vivo. The Journal of Immunology, 2011, 186: 25–31.

UV radiation is one of the most common environmental factors affecting human health. The UV wavelengths present in sunlight contribute significantly to the development of skin cancer (14), the most prevalent type of cancer found in the United States (15). Besides its carcinogenic effect, it is well-known that exposure to UV radiation is immunosuppressive, as demonstrated by the inhibition of cell-mediated immune reactions, such as contact and delayed type hypersensitivity (16, 17). A less well-recognized result of total body UV exposure is the suppression of T-dependent, but not T-independent, Ab formation (18–21). Although IL-10–producing T cells have been implicated in this process, the exact mechanism(s) leading to UV-induced suppression of Ab formation are not well defined.

After UV exposure, several cell populations are implicated in the process leading to immune suppression, including keratinocytes (22), macrophages (23), Langerhans cells (24), NKT cells (25), IL-10–secreting CD4+CD25+ T regulatory cells (26), and mast cells (27). In addition to their well-characterized role in type I hypersensitivity, mast cells have the potential to diminish inflammation and suppress immune responses (28). One of the first examples of mast cells playing a role in regulating adaptive immunity was the suppression of contact and delayed type hypersensitivity after UV exposure (27). Moreover, mast cell migration from UV-irradiated skin to the draining lymph node represents a mechanism by which an immune-suppressive signal is transmitted from the skin to the immune system (29). In addition, it has been shown that IL-10 produced by mast cells has the ability to limit inflammation in the skin (30), and it has been suggested that mast cell-derived IL-10 is essential for tolerance induction after UV exposure (31).

In this study, we examined the role of mast cells in the suppression of Ab formation. Exposing mice to UV radiation suppresses GC formation and affects Tfh cell function, whereas reconstituting the mast cell-deficient mice with wild type mast cells restores immune suppression. Our findings suggest that mast cells play a role in regulating adaptive immunity by affecting Tfh cell function, which is essential for GC formation and Ab production.
formation, Ab formation, the production of IL-21, and the expression of BCL-6 by Tfh cells. Suppression of Ab formation was blocked when UV-irradiated mice were treated with cromolyn, which blocks mast cell degranulation. No suppression of GC formation, IL-21 expression by Tfh cells, or activation of BCL-6 was noted in UV-irradiated mast cell-deficient mice. The suppressive effect was restored when mast cells were reconstituted with wild-type bone marrow-derived mast cells (BMMCs), but not when mast cells from IL-10−/− mice were used. These data demonstrate an immunoregulatory role for mast cells: suppressing GC formation by suppressing Tfh cell function.

Materials and Methods

Mice

Eight- to 10-week-old C57BL/6 wild type mice, mast-cell-deficient mice (KitW-sh/W-sh, IL-10−/− mice (B6;129P2-IL10tm1Wjy/J), and PGE2−/− mice (B6.129 [FVB]-Pgs2tm1(psp)wjy on the C57BL6 background were obtained from The Jackson Laboratory [Bar Harbor, ME]). The mice were maintained in facilities approved by the Association for Assessment and Accreditation of Laboratory Animal Care International, in accordance with current regulations and standards of the U.S. Department of Agriculture, Department of Health and Human Services, and National Institutes of Health. All animal procedures were reviewed and approved by the MD Anderson Cancer Center Animal Care and Use Committee.

To analyze the role of mast cells in UV-induced immune suppression, we reconstituted mast cell-deficient mice with BMMCs as described previously (29). Bone marrow cells were isolated from the femurs and tibias of 6- to 8-week-old C57BL/6, PGE2−/−, or IL-10−/− mice, and then cultured at a concentration of 10⁶ cells/ml in complete RPMI 1640 supplemented with murine recombinant IL-3 (10 ng/ml; Peprotech). Nonadherent cells were transferred to fresh culture medium twice a week for 4 to 6 wk, at which point >98% of viable cells were mast cells as verified by flow cytometry (CD117+ FcεRI+). A total of 1 × 10⁶ BMMCs were injected into eight sites underlining the dorsal skin of mast-cell-deficient mice. Six weeks later, the mice were exposed to UV radiation.

UV exposure and immunization

The shaved backs of the mice were exposed to an immunosuppressive dose of UV radiation (15 kJ/m² UVB; 290 to 320 nm) supplied by a 1000 W xenon arc solar simulator (Oriel Instruments, Stratford, CT), as described previously (32). The intensity and spectral output of the radiation source was measured with an Optronic model OL-754 scanning spectrophotometer (Optronic Laboratories, Orlando, FL). Seventy-two hours after irradiation, the mice were injected with 100 µg DNP-keyhole limpet hemocyanin (KLH) (Calbiochem, San Diego, CA) into the dermis on the lower back. Inguinal lymph nodes were removed 7 d postimmunization for Tfh cell analysis, at day 14 for GC analysis, and at day 28, sera were collected to measure Ag-specific IgG1 by ELISA.

To assess the effect of diosidum croymglogate (Sigma-Aldrich, St. Louis, MO) on Ab production, we modified a protocol described previously (33). In brief, C57BL/6 mice were injected i.p. with 10 mg/kg diosidum croymglogate for 5 d. Twelve hours after last injection, mice were UV-irradiated and immunized as described earlier.

ELISA

Maxisorp plates (Thermo Fisher Scientific, Waltham, MA) were coated overnight with 1 µg/ml KLH in coating buffer, blocked with blocking buffer, and incubated with sera serially diluted in blocking buffer. For detection, HRP-conjugated Ab specific for mouse IgG1 (Serotec, Raleigh, NC) was used. Signals were developed using substrate reagent, ended with stop solution, and recorded at 450 nm using a microplate reader. All solutions were from BD Bioscience (San Jose, CA).

Flow cytometry

Single-cell suspensions of lymph nodes were prepared by gentle mechanical disruption. Cells were suspended in PBS supplemented with 2% FBS and stained with the appropriate Ab. To detect Tfh cells, we stained cells with anti-CD4 (eBioscience, San Diego, CA), CD44 (eBioscience), BTLA (eBioscience), and biotin-CXCR5 (BD Bioscience), followed by allophycocyanin-streptavidin (BD Bioscience). GC B cells were labeled with CD19 (BD Biosciences), IgD (BD Bioscience), and PNA (Vector Laboratories, Burlingame, CA). Staining was measured with a FACS Calibur flow cytometer (BD Biosciences) and analyzed with FlowJo software (Tree Star, Ashland, OR).

Quantitative real-time PCR

Total RNA was extracted with TRIzol (Invitrogen, Carlsbad, CA) from sorted CD4+ CD44+ cells and further purified by treating with RNase-free RNA cleanup protocol (Qiagen, Germantown, MD). cDNA was reverse transcribed from total RNA using a high-capacity cDNA reverse transcription kit (Applied Biosystems, Carlsbad, CA). Twenty-five nanograms cDNA was subjected to quantitative real-time PCR using a sequence detector (Model ABI Prism 7500) and target mixes for IL-21, BCL-6, BLIMP-1, and β-actin (TaqMan Gene Expression Assay from Applied Biosystems). Cycle threshold (CT) values for IL-21, BCL-6, or BLIMP-1 were normalized to β-actin, as described previously (29), using the following equation: (1.8^ΔCt/β-actin – BCL6/β-actin – BCL6/β-actin – BLIMP1/β-actin) × 1000, where β-actin is the Ct of each actin control, BCL6 is the Ct of BCL-6, BLIMP-1 is the Ct of BLIMP-1, and 1000 is an arbitrary factor to bring all values to >1.

Immunofluorescence analysis

Lymph nodes were collected and immediately frozen in OCT tissue-freezing medium. Sections were cut to 5 µm thickness on a cryostat. Sections were stained with the following Abs: IgD (BD Bioscience), BCL-6 (Santa Cruz Biotechnology, Santa Cruz, CA), mast cell tryptase (Santa Cruz), IL-10 (Abcam, Cambridge, MA), goat IgG Alexa 488 (Invitrogen), rat IgG-Alexa 350 (Invitrogen), rabbit IgG-Alexa 594 (Invitrogen), biotinylated PNA (Vector Laboratories), and streptavidin-Alexa 594 (Invitrogen). Slides were mounted with Prolong Gold (Invitrogen). Images were obtained with an Olympus DP70 microscope (Olympus, Melville, NY).

Statistical analysis

Statistical difference between the control group and experimental groups was determined using a one-way ANOVA followed by Bonferroni’s multiple-comparison test (GraphPad Prism Software V4; GraphPad, San Diego, CA). Representative experiments are shown; each experiment was repeated independently at least three times.

Results

UV radiation blocks GC formation and Ab production

Although it is known that UV exposure can suppress Ab formation (18–21), little is known about the mechanisms involved, so we first determined whether UV exposure was able to suppress GC formation. C57BL/6 mice were exposed to a UV dose that suppressed delayed type hypersensitivity (15 kJ/m²), immunized with DNP-KLH, and 14 d postimmunization, GC formation was examined. We noted a decrease in GC formation as evidenced by a lack of PNA staining in the lymph nodes of mice exposed to UV and immunized with Ag (Fig. 1A). The fraction of PNA+ CD19+ IgD− cells in the lymph nodes of UV-irradiated and immunized mice was reduced when compared with what was found in nonirradiated but immunized control mice (Fig. 1B). GC formation was decreased when mice were immunized as early as 2 d post-UV irradiation (Fig. 1C; p < 0.001, UV + Ag versus Ag only).

The suppression of GC formation correlated with a diminished production of Ag-specific IgG1 (Fig. 1D), which was the only IgG subclass detected in the serum of DNP-KLH–immunized mice (Supplemental Fig. 1). These results indicate that GC formation is suppressed by UV exposure.

GC formation in mast cell-deficient mice are unaffected by UV radiation

Because of the growing evidence for the role of mast cells in regulating adaptive immune responses, we asked whether mast cells could be involved in UV-induced suppression of GC formation. A group of mice was pretreated with cromolyn, which prevents mast cell degranulation (34), daily for the 5 d before UV exposure. UV exposure suppressed Ag-specific IgG1 formation, and cromolyn treatment reversed this effect (Fig. 2A). To confirm this finding, we exposed mast cell-deficient mice (KitW-sh/W-sh) to UV radiation...
and then immunized them with DNP-KLH. Immunizing the mast cell-deficient mice resulted in Ab formation, and prior UV exposure did not suppress Ab formation in mast cell-deficient mice (Fig. 2B). Nor did we see any suppression of GC formation (Fig. 2C) or any depression of CD19+PNA+IgD− cell numbers when mast cell-deficient mice were exposed to UV and then immunized with Ag (Fig. 2D). These data indicate that mast cells regulate Ab production and GC formation.

UV exposure suppresses T follicular cell generation

Tfh cells are essential for GC formation (5). Next, we evaluated the effect of UV radiation on Tfh cell generation. Mice were exposed to UV and immunized with Ag. Seven days later, the inguinal lymph nodes were removed and a single-cell suspension was prepared. We gated on the CD4+ CD44+ cells and used expression of CXCR5 and BTLA to identify Tfh cells (Fig. 3A). As expected, we saw an upregulation of Tfh cells in the lymph nodes of immunized mice (Ag only) compared with the nonimmunized controls (no Ag). Prior UV exposure suppressed the Ag-induced upregulation of CXCR5+ and BTLA+ on CD4+ CD44+ cells (Fig. 3B, C). IL-21 acts in an autocrine fashion promoting Tfh cell survival or activation, or both (12, 13). We next tested whether UV radiation could alter IL-21 expression by the CD4+ CD44+ cells. Lymph nodes were removed from UV-irradiated Ag-immunized mice or from unirradiated Ag-immunized control mice. The CD4+ CD44+ cells were then isolated by cell sorting. Prior UV exposure resulted

FIGURE 1. GC formation is blocked by UV exposure. C57BL/6 mice were exposed to UV, and 3 d later were immunized with DNP-KLH. Fourteen days after immunization, draining lymph nodes were collected and analyzed for GC formation. A, Representative immunofluorescence image from lymph nodes sections stained with anti-IgD (blue) and PNA (red). Original magnification ×100. Scale bar, 200 μm. B, Representative flow cytometric dot plot of IgD− PNA+ GC B cells gated on total CD19+. C, Mice were immunized at different days after UV exposure, and GC B cells were analyzed at day 14 by flow cytometry. Data are expressed as mean ± SEM. n = 3 mice/group. *p < 0.001 versus Ag only. D, Mice were bled 28 d postimmunization, and KLH-specific IgG1 was measured by ELISA. The data are expressed as means ± SEM. n = 5 mice/group. *p < 0.05 compared with mice immunized with DNP-KLH. Representative experiments are shown; each experiment was repeated independently at least three times.

FIGURE 2. Mast cell-deficient mice are resistant to UV-induced suppression of GC formation. A, C57BL/6 mice received five daily injections of cromolyn (10 mg/kg i.p.) and then were exposed to UV radiation. Three days later, they were immunized; 28 d later, serum levels of anti-KLH IgG1 were measured. Data are expressed as means ± SEM. n = 5 mice/group. B, Mast cell-deficient KitW−sh/W−shmice were UV-irradiated and immunized 3 d later. IgG1 specific for KLH was evaluated 28 d after immunization. The graph shows means ± SEM. n = 5 mice/group. C, KitW−sh/W−shmice were exposed to UV and 3 d later were immunized with DNP-KLH. Fourteen days after immunization, draining lymph nodes were collected and analyzed for GC formation. A representative immunofluorescence image from lymph node sections stained with anti-IgD (blue) and PNA (red) is shown. Original magnification ×100. Scale bar, 200 μm. D, Mice were immunized 3 d after UV exposure and GC B cells analyzed 14 d later by flow cytometry. E, Flow cytometric dot plot of IgD− PNA+ GC B cells gated on total CD19+ from mast cell-deficient mice. Representative experiments are shown; each experiment was repeated independently at least three times.
were measured, we observed suppression of IL-21 expression when cells were stimulated with DNP-KLH in vitro, and IL-21 mRNA levels were measured. We observed no difference in the number of pro- lactinating CD4 cells (Supplemental Fig. 2 A) regardless of treatment, nor was any difference in IFN-γ production measured. We detected IL-10–producing mast cells 24 h after UV exposure in the inguinal lymph nodes that drained the UV-irradiated dorsal skin, but not in non-skin draining popliteal lymph nodes (Supplemental Fig. 3). We then asked whether the generation of Tfh cells is altered in mast cell-deficient mice. First, we observed that the expression of CXCR5 and BTLA on CD4+ CD44+ cells was similar in UV-irradiated and nonirradiated mast cell-deficient mice (Fig. 4A, 4B). Similarly, sorted CD4+ CD44+ cells from both groups of mice expressed almost identical levels of IL-21 mRNA (p > 0.05, Ag only versus UV + Ag; Fig. 4C). Finally, CD4+ CD44+ sorted cells from both groups showed similar BCL-6/BLIMP1 ratios (Fig. 4D; p > 0.05, Ag only versus UV + Ag). These results indicate that Tfh cells are generated in mast cell-deficient mice and are unaffected by prior UV exposure.

**Mast cell-deficient mice are resistant to UV-induced suppression of Tfh cell generation**

Next we asked whether the generation of Tfh cells is altered in mast cell-deficient mice. First, we observed that the expression of CXCR5 and BTLA on CD4+ CD44+ cells was similar in UV-irradiated and nonirradiated mast cell-deficient mice (Fig. 4A, 4B). Similarly, sorted CD4+ CD44+ cells from both groups of mice expressed almost identical levels of IL-21 mRNA (p > 0.05, Ag only versus UV + Ag; Fig. 4C). Finally, CD4+ CD44+ sorted cells from both groups showed similar BCL-6/BLIMP1 ratios (Fig. 4D; p > 0.05, Ag only versus UV + Ag). These results indicate that Tfh cells are generated in mast cell-deficient mice and are unaffected by prior UV exposure.

**IL-10 produced by mast cells is critical for UV-induced suppression of GC formation**

The immunoregulatory cytokine IL-10 plays a critical role in UV-induced immune suppression (22). We detected IL-10–producing mast cells 24 h after UV exposure in the inguinal lymph nodes that drained the UV-irradiated dorsal skin, but not in non-skin draining popliteal lymph nodes (Supplemental Fig. 3). We then asked whether the generation of Tfh cells is altered in mast cell-deficient mice. First, we observed that the expression of CXCR5 and BTLA on CD4+ CD44+ cells was similar in UV-irradiated and nonirradiated mast cell-deficient mice (Fig. 4A, 4B). Similarly, sorted CD4+ CD44+ cells from both groups of mice expressed almost identical levels of IL-21 mRNA (p > 0.05, Ag only versus UV + Ag; Fig. 4C). Finally, CD4+ CD44+ sorted cells from both groups showed similar BCL-6/BLIMP1 ratios (Fig. 4D; p > 0.05, Ag only versus UV + Ag). These results indicate that Tfh cells are generated in mast cell-deficient mice and are unaffected by prior UV exposure.

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whether mast cell-derived IL-10 is mediating UV-induced suppression of humoral immunity. Mast cell-deficient mice that were reconstituted by injecting BMMCs from either wild type or IL-10–deficient animals into the skin showed a similar mast cell density in the reconstituted area when compared with the density of mast cells found in wild type mice (Supplemental Fig. 4A). In the absence of UV exposure, reconstituting KitW-sh/W-sh mice with normal or IL-10–deficient BMMCs did not affect Ab production or Tfh cell generation (Supplemental Fig. 4B, 4C). When mast cell-deficient mice were reconstituted with normal BMMCs and then UV-irradiated, a significant reduction in IL-21 mRNA expression by CD4+ CD44+ cells was observed (Fig. 5A; p < 0.05, UV + BMMC B6 versus Ag only). These cells also expressed a diminished BCL-6/BLIMP-1 ratio compared with cells isolated from the nonirradiated immunized control mice (Fig. 5B; p < 0.001, UV + BMMC B6 versus Ag only) and exhibited a decreased expression of CXCR5 and BTLA on CD4+ CD44+ cells (Fig. 5C). In contrast, IL-21 expression (Fig. 5A), the BCL-6/BLIMP-1 ratio (Fig. 5B), and the numbers of Tfh cells (Fig. 5C) were not suppressed in UV-irradiated mast cell-deficient mice reconstituted with IL-10−/− BMMCs (p > 0.05 versus Ag only control). Similarly, the numbers of Tfh cells was unaltered in mice reconstituted with IL-10−/− BMMCs, compared with the positive control (Ag only; Fig. 5D). We also found no suppression of GC formation when IL-10–deficient BMMC reconstituted mice were exposed to UV radiation (Fig. 5E). Similarly, no suppression of Ab production was observed when IL-10−/− BMMCs were used to reconstitute UV-irradiated mast cell-deficient mice (Fig. 5F). As a control, another group of mast cell-deficient mice was reconstituted with BMMCs isolated from PG endoperoxidase synthase 2-deficient (PGE2−/−) animals. GC formation and Tfh cell activation in these mice was suppressed by prior UV exposure, indicating that mast cell-derived PGE2 is not involved in suppressing GC formation (Supplemental Fig. 5). Taken as a whole, these results indicate that mast cells can suppress humoral immunity. Furthermore, they indicate that mast cell-derived IL-10 is responsible for suppressing GC formation, Tfh cell activation, and Ab production in vivo.

**Discussion**

In these experiments, we used the environmentally relevant carcinogen UV radiation to activate immune suppression. The immunosuppressive properties of UV radiation first became evident in experiments studying the immunobiology of UV-induced skin cancers (36). Since that time, the immunosuppressive properties of UV radiation have been confirmed many times, in both mice and humans (35). Most of the studies using UV as an immunosuppressive agent have examined its effects on cell-mediated immunity (35). Although it is also known that UV exposure suppresses humoral immunity (18–20), little is understood about the mechanisms involved. Recent studies have shown that Tfh cells are the cru-

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**FIGURE 5.** Mast cell-derived IL-10 is crucial for UV-induced humoral immune suppression. Mast cell-deficient mice or mast cell-deficient mice reconstituted with wild type (B6) or IL-10−/− BMMCs were exposed to UV and immunized with Ag. Lymph node CD4+ CD44+ cells were isolated by cell sorting 7 d postimmunization (A). IL-21 mRNA expression by CD4+ CD44+ cells. *p < 0.05 versus mast cell-deficient mice immunized with DNP-KLH (Ag only). B, Ratio of BCL-6/BLIMP-1 expression by CD4+ CD44+ cells. *p < 0.001 versus mast cell-deficient mice immunized with DNP-KLH (Ag only). C, Mast cell-deficient mice and BMMC reconstituted mice were exposed to UV, and 3 d later were immunized with Ag. Tfh cells were analyzed 7 d later by flow cytometry. *p < 0.05 versus mast cell-deficient mice immunized with DNP-KLH (Ag only). D, Representative flow cytometric dot plot of CXCR5+ BTLA+ Tfh cells gated on total CD4+ CD44+. E, Representative immunofluorescence image from lymph node sections stained with anti-IgD (green) and BCL-6 (red). Original magnification ×100. Scale bar, 200 μm. F, Mice were bled 28 d postimmunization, and KLH-specific IgG1 was measured by ELISA. Data are expressed as means ± SEM. n = 5 mice/group. *p < 0.05 compared with nonirradiated immunized (Ag only) mice. Representative experiments are shown; each experiment was repeated independently at least three times.
cial cells that provide costimulatory signals and cytokines necessary for B cell activation, GC formation, and Ab production (37). In this report, we provide evidence that UV radiation suppresses Ab production by inhibiting GC development as early as 48 h post-irradiation. Furthermore, we observed a decreased fraction of CXCR5+ CD4+ cells and decreased production of IL-21 by activated T cells isolated from UV-exposed mice. We hypothesize that the reduction in Tfh cell number is due to decreased levels of IL-21, which works in an autocrine manner to promote Tfh cell activation and the help necessary for GC formation and Ab production (13). However, because many other cell types in addition to Tfh produce IL-21 (37), we could not rely solely on IL-21 as an indicator of Tfh cell activation. The recent identification of BCL-6 as the master transcription factor (9) and BLIMP-1 as the repressor of Tfh cell differentiation (10) allowed us to more precisely identify the effect of UV exposure on Tfh cell activation. Our results show that CD4+ CD44+ cells from UV-irradiated Ag-immunized mice had a depressed BCL-6/BLIMP-1 ratio compared with what was observed with cells from nonirradiated immunized control mice, indicating that UV radiation targets Tfh cell generation.

We also demonstrated that mast cells, and mast cell-derived IL-10, play a role in suppressing Tfh cell generation, IL-21 expression, GC formation, and Ab production. These findings add to the appreciation of the important role IL-10 plays in UV-induced immune suppression and UV-induced skin cancer formation (35). How mast cells alter T activation in the lymph node is not completely understood. Others have suggested that activated mast cells can act as APCs, form an immunological synapse with T cells, and directly affect their activation (38). Evidence also indicates that cytokines produced by activated mast cells (IL-4) can induce Th2 differentiation (39). Our studies further support the important role that mast cell-derived cytokines play in immune regulation by demonstrating that mast cell-derived IL-10 is the critical immunosuppressive factor that blocks Tfh cell generation, GC formation, and Ab production.

Although it is well-known that UV radiation only is absorbed in the top layers of the skin, it can clearly effect GC formation in skin-draining lymph nodes. The data presented in this study support the concept that dermal mast cells transmit the immunosuppressive signal from the skin to the lymph nodes, although the exact mechanism is not entirely clear. Two potential mechanisms may be involved. Previously, we reported that UV triggers mast cell migration from the skin to the lymph node, and agents to block mast cell migration (i.e., CXCR4 antagonists) block the induction of immune suppression (29). We suggest that dermal mast cells are triggered by UV exposure to leave the skin and migrate to the draining lymph nodes, where they release IL-10 and suppress GC formation. Another mechanism that activated mast cells can use to deliver immune regulatory signals from the skin to distant lymph nodes is via the release of small, spherical, cytokine-containing, heparin-containing, heparin-based particles. Kunder and colleagues (40) recently presented data demonstrating that these microparticles can enter lymphatic vessels, migrate to draining lymph nodes, and release bioactive TNF. It is possible that UV-activated dermal mast cells release IL-10–containing heparin granules, which enter the lymphatics, migrate to the draining lymph nodes, and suppress Tfh cell function.

Although the results presented in this study indicate that mast cells suppress Ab formation, it should be pointed out that a report by McLachlan and colleagues (41) indicates that mast cells can also serve as adjuvants for Ab formation. Their data indicate that administration of mast cell activators with Ag induces mast cells to secrete TNF, which promotes the migration of dendritic cells to the lymph nodes and enhances Ab production. The critical difference between their findings and the data presented in this study appears to be the agents that are used to activate the mast cell. McLachlan et al. (41) used compound 48/80, a classic mast cell activator, to induce TNF release and promote the immune response. When we reconstituted mast cell-deficient mice with normal BMMCs and simply immunized the animals, we saw no effect, positive or negative, on Ab formation (Supplemental Fig. 4). However, when we exposed mice to a classic immunosuppressive agent, UV radiation, the mast cells were activated to release IL-10 and suppress Tfh cell function. These observations support the idea that mast cells are “tunable” regulatory cells; that is, depending on the environment and the signals they receive, they can either promote or suppress the immune response (42).

In conclusion, our results show, to our knowledge, a new function for mast cells. We find that mast cells suppress Tfh cell generation, the formation of GCs, and Ab production via the production of IL-10. These findings support the growing appreciation of mast cells as immunoregulatory elements. We suggest that mast cells, in addition to blocking classic cell-mediated immune reactions (i.e., delayed-type hypersensitivity) (27, 29, 30), also regulate humoral immune reactions by negatively affecting Tfh cell function.

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Disclosures
The authors have no financial conflicts of interest.

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