

Human mast cells drive memory CD4⁺ T cells toward an inflammatory IL-22⁺ phenotype

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Background: Mast cells are key components of the skin microenvironment in psoriasis, yet their functional role in this T-cell-mediated inflammatory disorder remains to be elucidated.

Objective: To define the impact of T-cell/mast-cell cognate interactions on the cytokines produced by T_H cells.

Methods: We used human primary mast cells and effector/memory CD4⁺ T cells for *in vitro* coculture experiments, and we analyzed T_H cells responses by using cytometry. CD4⁺ T-cell/mast-cell conjugates in skin lesions from patients with psoriasis were analyzed by using 3-color immunohistochemistry and confocal microscopy.

Results: We show that IFN- γ -primed human mast cells formed productive immunologic synapses with antigen-experienced CD4⁺ T cells. These interactions promoted the generation of T_H22 and IL-22/IFN- γ -producing T_H cells from the circulating memory CD4⁺ T-cell pool via a TNF- α /IL-6-dependent mechanism. An analysis of human psoriatic skin biopsies showed a rich infiltrate of IL-22⁺CD4⁺ T cells frequently found in contact with mast cells. Moreover, most of these mast-cell-conjugated lymphocytes coexpressed IFN- γ , suggesting that IL-22⁺IFN- γ ⁺ CD4⁺ T cells are generated *in vivo* on interaction with mast cells.

Conclusions: Our findings identify human mast cells as functional partners of T_H cells, shaping their responses toward IL-22 production. (J Allergy Clin Immunol 2013;■■■:■■■-■■■.)

Key words: Mast cells, T_H cells, IL-22, immunologic synapse, inflammation, psoriasis

Naive CD4⁺ T-cell differentiation into T_H cells occurs in secondary lymphoid organs in response to signals provided by professional antigen-presenting cells (APCs). Effector/memory T_H cells infiltrate inflamed tissues, where they receive additional signals from the microenvironment and resident APCs. The plasticity of antigen-experienced CD4⁺ T cells might allow them to

Abbreviations used

APC:	Antigen-presenting cell
CLA:	Cutaneous lymphocyte-associated antigen
DC:	Dendritic cell
hMC:	Human mast cell
hMC ^{IFN-γ} :	hMC treated for 72 hours with IFN- γ
IHC:	Immunohistochemistry
SAg:	Superantigen
SCF:	Stem cell factor

adapt their response to the local immunologic milieu.¹⁻⁴ While the role of professional APCs in T_H differentiation has been thoroughly investigated, the impact that tissue-resident immune cells might have on T_H responses after antigen reexposure is less understood.

Mast cells are widely distributed in tissues and are especially enriched in regions exposed to the external environment such as skin, gastrointestinal tract, and airways.⁵ In the dermis, they are found to be among the most frequent leukocytes under steady-state conditions (approximately 75-100 cells/mm²).⁶ Moreover, their number has been reported to increase in skin diseases such as psoriasis and atopic dermatitis.^{7,8}

Being strategically located, mast cells are among the first immune cells to encounter antigens or incoming pathogens in tissues.^{5,9-11} Although mast cells are often associated with allergic processes, several studies have demonstrated that their participation in the immune response extends far beyond their well-known role in allergy.^{5,11,12}

We have recently shown that IFN- γ -primed mouse mast cells can present antigenic determinants to CD4⁺ T cells, resulting in the formation of functional immunologic synapses.¹³ Cognate mast cells were shown to form productive interactions only with previously activated T cells and not with their naive counterpart, indicating that, at least in mouse models, mast cells might selectively cooperate with tissue-infiltrating CD4⁺ T cells exhibiting an effector/memory phenotype. Nevertheless, the impact of mast-cell-mediated antigen presentation on the outcome of human T_H cell biological responses and its possible link to human disease remain to be elucidated.

Here, we show that mast cells establish synaptic-like contacts with CD4⁺ T cells in inflamed skin from patients with psoriasis and that human peripheral blood-derived mast cells primed with IFN- γ form productive immunologic synapses with antigen-experienced CD4⁺ T cells. This cooperation induces the generation of T_H22 and IL-22⁺IFN- γ ⁺ T_H cell subsets from circulating memory CD4⁺ T cells. We also show that in psoriatic skin, CD4⁺ T_H cells in contact with mast cells frequently express IL-22 either alone or in combination with IFN- γ . Our findings identify human mast cells (hMCs) as functional partners of T_H cells, shaping T_H cell responses toward IL-22 production.

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METHODS

Mast-cell culture and priming

CD133⁺ cells were seeded in Iscove's modified Dulbeccos medium with GlutaMAX-I, sodium pyruvate, 2-mercaptoethanol, 0.5% BSA, insulin-transferrin-selenium (all from Life Technologies, Carlsbad, Calif), ciprofloxacin (10 µg/mL, Sigma-Aldrich, St Louis, Mo), human IL-6 (50 ng/mL, Peprotech, Rocky Hill, NJ), and 3% supernatant of Chinese hamster ovary transfectants secreting murine stem cell factor (SCF; a gift from Dr P. Dubreuil, Marseille, France). Cells were cultured for 2 months and tested phenotypically (tryptase⁺, CD117⁺, FcεRI⁺) and functionally (β-hexosaminidase release in response to FcεRI cross-linking) before use for experiments. hMCs were primed in the culture medium with IFN-γ (50 ng/mL, eBiosciences, San Diego, Calif) for 72 hours and referred to as hMC^{IFN-γ}.

Memory CD4⁺ T-cell differentiation and coculture experiments

A total of 5×10^4 freshly purified memory CD4⁺ T cells were incubated for 6 days in 96-well U-bottom plates, with 5×10^4 APCs pulsed or unpulsed with a cocktail of superantigen (SAg; 50 ng/mL TSST-1, SEA, SEB, SEE, and Sec-1, Toxin Technology, Sarasota, Fla) or with 5×10^4 anti-CD3/CD28-coated beads (Life Technologies) in RPMI 1640 supplemented with 10% serum replacement medium (knockout medium, Life Technologies), GlutaMAX-I, sodium pyruvate, 2-mercaptoethanol, ciprofloxacin (10 µg/mL, Sigma-Aldrich), and 1% SCF. At day 3, 20 IU/mL of human IL-2 (Proleukin, Chiron, Emeryville, Calif) was added. At day 6, cells were processed for intracellular cytokine staining.

Patients and tissue staining

The patients (4 men and 3 women; mean age 45 years) had moderate to severe psoriasis; their Psoriasis Area Severity Index score ranged between 14 and 44 (mean 24.3). Psoriatic skin samples fixed in 10% buffered formalin (n = 6) or Dubosq-Brazil (n = 1) and nonlesional skin samples as controls (n = 9) were retrieved from the tissue bank of the Department of Pathology at the Purpan Hospital between 2010 and 2011. The use of these samples was approved by the institutional review board of the Toulouse University Hospital. Fixed and embedded in paraffin, specimens were processed for routine histopathologic examination. Three-micrometer-thick sections were stained with hematoxylin and eosin.

For immunohistochemistry (IHC), 3-µm-thick sections were tested by using a Ventana BenchMark XT immunostainer (Ventana). Double staining was performed with anti-mast cell tryptase (FL-275, Santa Cruz Biotechnology) or CD117 (A4502, DAKO) and anti-CD4 (1F6, DAKO).

Triple staining was performed first with anti-IL22 (polyclonal, Thermo Scientific) and CD117 (A4502, DAKO) by using a Ventana Benchmark XT immunostainer (Ventana, Tucson, Ariz) and then adding manually anti-CD4 (1F6, DAKO) with the VECTASTAIN Universal ABAP Kit (Vector Blue AP substrate; blue). Samples were scored by an expert pathologist (C.L.).

For confocal microscopy analysis, 3-µm-thick sections were pretreated by microwave incubation in TRIS-EDTA buffer (pH 9.0) and then in 0.1 mol/L sodium citrate (pH 6.0). Samples were then permeabilized and stained overnight with antibodies against CD4, tryptase, and IL-22. Secondary antibodies were applied for 2 hours at room temperature.¹⁴ Fluorescence was analyzed with a Zeiss LSM 710 confocal microscope, and images were processed and quantified by using Zen software.

Statistical analysis

Unpaired or paired Student *t* tests were used for comparing 2 groups. Data are expressed as means ± SEM (*P* value range is indicated: **P* < .05, ***P* < .01, and ****P* < .001). Flow cytometric profiles and histology images are representative of repeated experiments.

A complete description of the methods is available in this article's Online Repository available at www.jacionline.org.

RESULTS

hMCs establish immunologic synapses with CD4⁺ T cells and induce their proliferation *in vitro*

We investigated the nature of CD4⁺ T-cell/mast-cell interactions *in vitro*. Human CD133⁺ peripheral blood precursors were cultured in the presence of SCF and IL-6 as described.¹⁵ After 2 months of culture, the cell population was negative for CD14 (not shown) and harbored phenotypical and functional characteristics of mature hMCs (see Fig E1 in this article's Online Repository available at www.jacionline.org). Cells expressed tryptase but not chymase and were functionally close to connective tissue mast cells as they responded to substance P and compound 48/80 (Fig E1). Similar to their mouse counterparts,¹³ hMC^{IFN-γ} expressed MHC class II and costimulatory molecules CD54 and CD80 (see Fig E2 in this article's Online Repository available at www.jacionline.org). However, treatment with IL-4 either alone or in combination with IFN-γ did not affect MHC class II and costimulatory molecule expression (data not shown). We showed that hMC^{IFN-γ} loaded with the bacterial SAg TSST-1 were able to induce polyclonal CD4⁺Vβ2⁺ T-cell line proliferation (see Fig E3, A, in this article's Online Repository available at www.jacionline.org). We next investigated the immunologic synapse formation to directly visualize the productive interaction between CD4⁺ T cells and mast cells. While under TSST-1-unloaded conditions, CD3ε and phosphotyrosine (pTyr) were not enriched at the T-cell/mast-cell contact site, the interaction of T cells with TSST-1-loaded hMC^{IFN-γ} resulted in CD3ε and pTyr enrichment at the synaptic area (Fig E3, B and C). In parallel time-lapse microscopy experiments, CD4⁺Vβ2⁺ T cells interacting with TSST-1-pulsed hMC^{IFN-γ} exhibited sustained [Ca²⁺]_i increase, a key signaling event in T-cell activation (see Video E1 in this article's Online Repository at www.jacionline.org). Taken together, these results indicate that IFN-γ-primed hMCs are capable of forming immunologic synapses with antigen-experienced CD4⁺ T cells as well as stimulating these cells.

Memory CD4⁺ T cells produce IL-22 after interaction with cognate hMCs

We next investigated the functional outcome of hMC^{IFN-γ} interaction with freshly isolated memory CD4⁺ T cells by analyzing cytokines produced by T_H cells. Memory CD4⁺CD45RO⁺CD45RA⁻ T cells were isolated from the peripheral blood of healthy human donors and cultured for 6 days with (1) anti-CD3/CD28-coated beads to trigger TCR and CD28 in the absence of polarizing factors such as cytokines and APC accessory molecules; (2) LPS-primed monocyte-derived dendritic cells (DC^{LPS}), as professional APC; or (3) hMC^{IFN-γ}. Both DC^{LPS} and hMC^{IFN-γ} were pulsed with a cocktail of bacterial SAg to activate a substantial fraction of the polyclonal memory T-cell population.¹⁶

As shown in Fig 1, A, mast cells were excluded from the fluorescence-activated cell sorting analysis on the basis of their CD117 expression (similarly for DC^{LPS} on the basis of CD11c expression; not shown). Stimulation with hMC^{IFN-γ} induced a substantial fraction of IFN-γ-producing CD4⁺ T cells, comparable to that observed in cultures with DC^{LPS} (Fig 1, B and C). At the individual cell level, mast-cell-activated T cells exhibited a brighter IFN-γ staining when compared with DC^{LPS}-activated T cells (Fig 1, B). However, the number of IFN-γ-producing cells and the amount of secreted

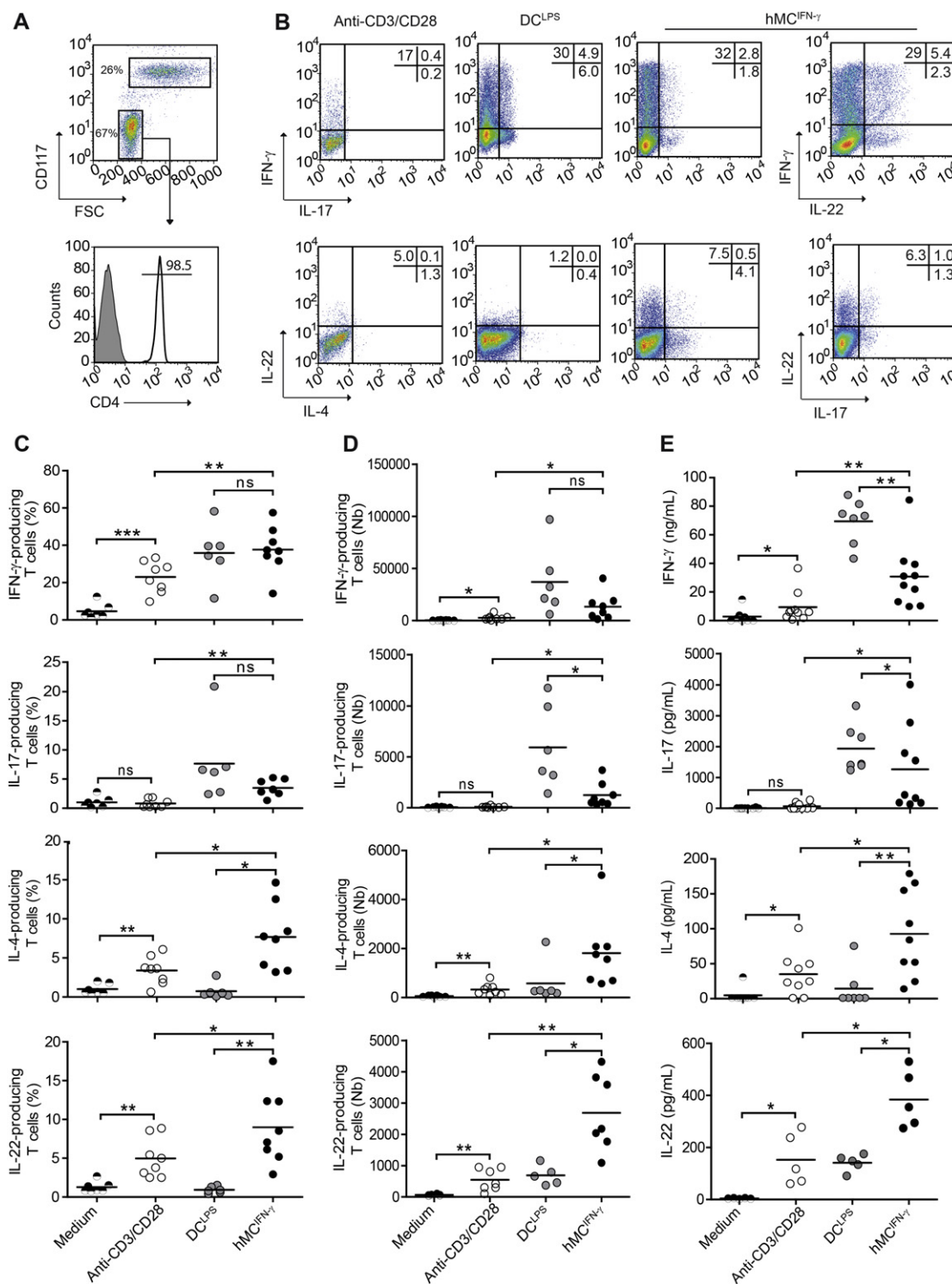


FIG 1. Memory T cell/hMC^{IFN-γ} cognate interactions result in the induction of IL-22-producing T_H cells. Production of IFN-γ, IL-17, IL-4, and IL-22 by memory CD4⁺ T cells. **A**, Gating strategy. **B-D**, Representative dot plots (Fig 1, B), frequency (Fig 1, C), and numbers (Fig 1, D) of CD4⁺ T cells producing indicated cytokines. **E**, Cytokine production in the supernatant of cocultures. Each symbol represents 1 donor. Two-tailed, paired *t* test. **P* < .05, ***P* < .01, and ****P* < .001. ns, Not significant.

IFN-γ were lower in hMC^{IFN-γ}-stimulated T-cell cultures (Fig 1, D and E). Stimulation with anti-CD3/CD28-coated beads resulted in a weaker induction of IFN-γ production (Fig 1, B-E).

We observed a significant induction of IL-17-producing cells after stimulation with DC^{LPS} and hMC^{IFN-γ}, which was not observed after stimulation with anti-CD3/CD28-coated beads. hMC^{IFN-γ} induced a stronger IL-4 response as

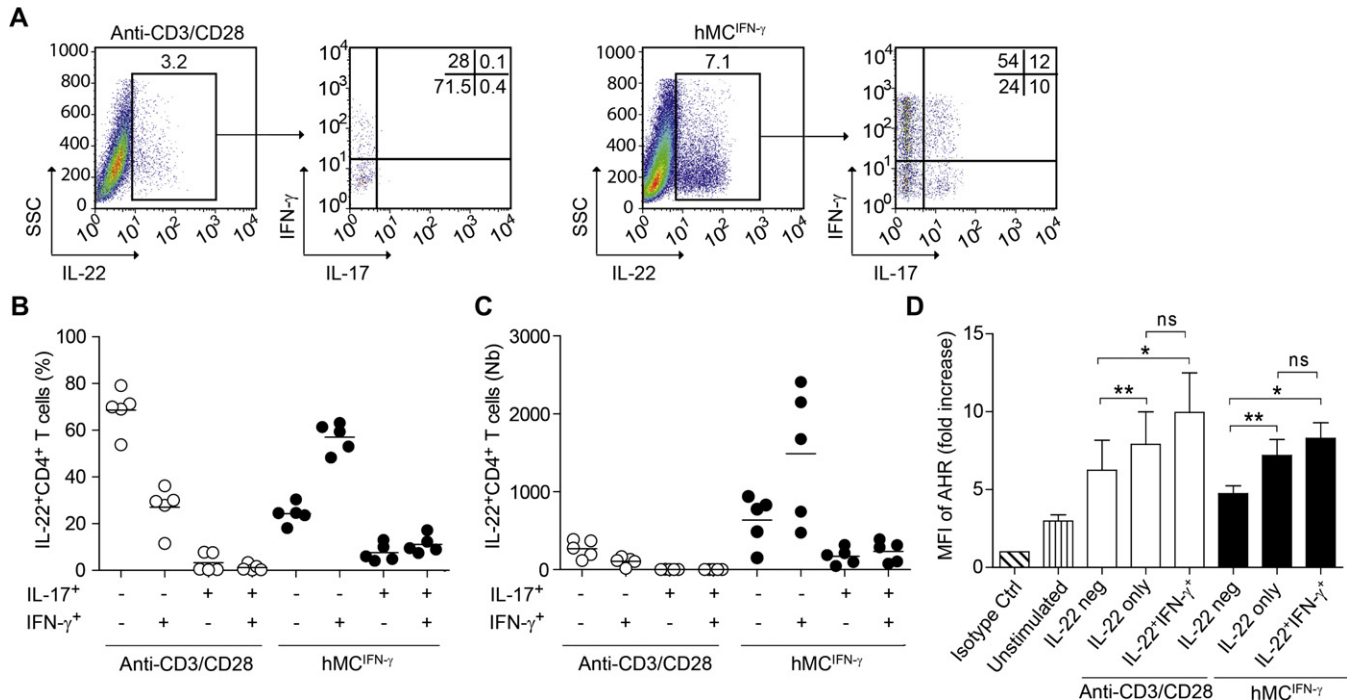


FIG 2. hMC^{IFN-γ} induce T_H22 and IFN-γ⁺IL-22⁺ T_H cells. **A**, IL-17⁺ and IFN-γ⁺ producing cells among IL-22⁺CD4⁺ T cells. **B** and **C**, Representative dot plots of frequency (Fig 2, **B**) and numbers (Fig 2, **C**) of IL-22⁺CD4⁺ T cells expressing IL-17, IFN-γ, or both. Each symbol represents 1 donor. **D**, Fold increase in AHR mean fluorescence intensity (MFI) over the isotype control. Mean ± SEM of 4 independent experiments from 2 donors. Two-tailed, paired t test, **P* < .05 and ***P* < .01. AHR, Aryl hydrocarbon receptor; ns, not significant.

compared with both DC^{LPS} and anti-CD3/CD28-coated beads (Fig 1, *B-E*).

With regard to IL-22 production, anti-CD3/CD28-coated bead stimulation resulted in a detectable induction of IL-22-producing cells. Conversely, DC^{LPS} did not support IL-22 production. Interestingly, hMC^{IFN-γ} induced an unexpectedly strong IL-22 response in the memory CD4⁺ T-cell population. Both the percentage and the number of IL-22-producing cells were significantly increased compared with control stimulations, reaching up to 10% to 15% of IL-22⁺ cells and resulting in a substantial release of IL-22 in the culture medium (Fig 1, *B-E*). The above results show that on interaction with mast cells, memory CD4⁺ T cells are activated to produce a broad range of cytokines and, in particular, relatively high amounts of IL-22.

hMC functional interaction with memory CD4⁺ T cells results in T_H22 and IFN-γ⁺/IL-22⁺ T_H cells

To deepen our characterization of IL-22-producing T cells generated in the presence of mast cells, we investigated their cytokine profile and homing potential. After stimulation with anti-CD3/CD28-coated beads, more than 70% of the relatively small fraction of IL-22⁺ T cells were both IL-17 and IFN-γ negative (IL-22 only; Fig 2, *A* and *B*). Conversely, hMC^{IFN-γ} stimulation led to a heterogeneous profile with a major population of double-positive IFN-γ⁺IL-22⁺ T cells (corresponding to >50% of total IL-22⁺ T cells) and a population of “IL-22-only”-producing cells (25%-30% of total IL-22-positive cells; Fig 2, *A-C*). In parallel experiments, the hMC^{IFN-γ} stimulation of peripheral CD4⁺ T cells isolated from PBMCs on the basis of their expression of the cutaneous lymphocyte-associated antigen (CLA)^{17,18}

led to the emergence of these 2 cell subsets (see Fig E4 in this article's Online Repository available at www.jacionline.org). This result indicates that a skin homing CLA⁺CD4⁺ T-cell population (composed of memory and effector cells) differentiates into similar IL-22⁺ T cell subsets when stimulated with hMC^{IFN-γ}.

We also measured the expression of the aryl hydrocarbon receptor transcription factor (which favors IL-22 production^{19,20}) by using fluorescence-activated cell sorting analysis. As shown in Fig 2, *D*, the IL-22⁺ T-cell populations exhibited an increased expression of AHR.

We next analyzed the expression of the chemokine receptors CCR4, CCR6, and CCR10. After the stimulation of both anti-CD3/CD28-coated beads and hMC^{IFN-γ}, most IL-22⁺CD4⁺ T cells were CCR6 positive (see Fig E5 in this article's Online Repository available at www.jacionline.org). Among these cells, 70% to 80% also expressed the skin homing receptor CCR4 and only a small fraction was found to be CCR10 positive (Fig E5). These results indicated that regardless of the stimulation conditions, IL-22-producing cells harbored comparable homing potential.

Taken together, the above results indicate that the stimulation of memory T cells with SAg-presenting mast cells results in the induction of 2 major IL-22-producing cell populations: one representing pure T_H22 cells and the other, which we defined as IL-22⁺/IFN-γ T_H cells, exhibiting a mixed T_H1/T_H22 phenotype.

Mast cells induce IL-22-producing T_H cells via a TNF-α/IL-6-dependent mechanism

We next investigated the mechanisms that hMC^{IFN-γ} use to induce IL-22 production in T_H cells. We focused on IL-6 and TNF-α that have been shown to be required for the commitment

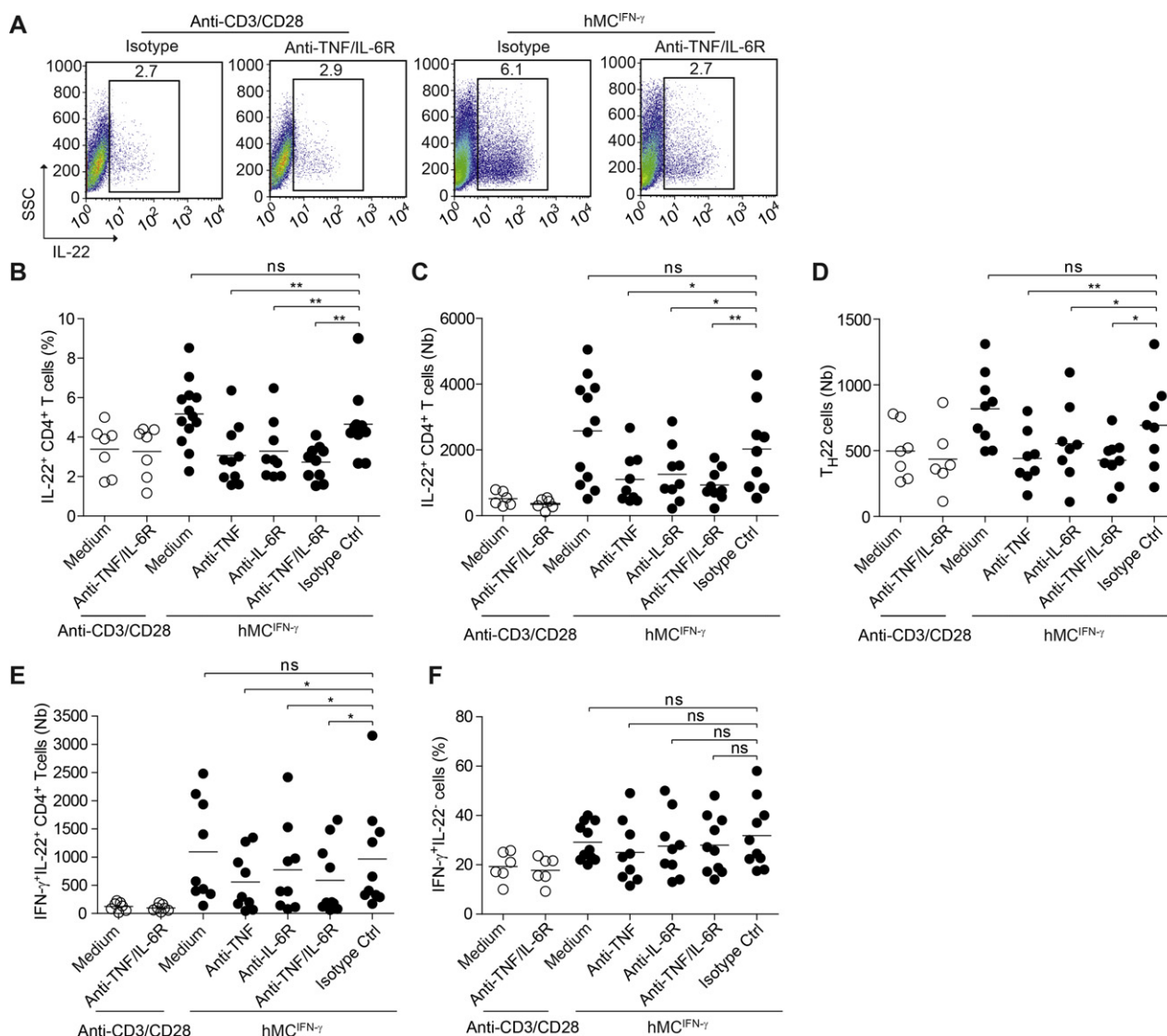


FIG 3. hMC^{IFN-γ} induce IL-22 production via a TNF- α /IL-6-dependent mechanism. The effect of the IL-6 receptor and TNF- α -neutralizing antibodies on the frequency (A, B, F) and number (C-E) of cells producing IL-22 and/or IFN- γ is shown. Each symbol represents 1 donor. Two-tailed, paired *t* test, **P* < .05 and ***P* < .01. ns, Not significant.

of naive CD4⁺ T cells to IL-22 production.²¹ The IL-6 receptor and/or TNF- α -blocking antibodies had no significant effect on the low but detectable percentage and number of IL-22-producing cells on CD3/CD28 stimulation (Fig 3). Conversely, on hMC^{IFN-γ} stimulation, blocking antibodies used separately or simultaneously induced a significant decrease in the percentage and number of IL-22-producing cells (Fig 3, A-C) including TH22 (Fig 3, D) and IL-22⁺/IFN- γ ⁺ TH cell subsets (Fig 3, E). The same treatment did not affect the percentage of IFN- γ -producing cells, indicating that blocking antibodies did not interfere with productive TCR engagement and T-cell activation (Fig 4, F). These results extend the notion that IL-6 and TNF- α are key cytokines involved in IL-22 induction in CD4⁺ T cells.

Human psoriatic skin is enriched in IL-22⁺CD4⁺ T-cell/mast-cell conjugates

To investigate whether interactions between hMCs and TH cells might take place in tissues, we focused on psoriasis, a skin

inflammatory disorder characterized by abundant mast-cell and T-cell infiltrates and by augmented IL-22 production.²²⁻²⁴ By using IHC, we examined the number and localization of CD4⁺ T cells and mast cells in lesional skin biopsies from patients with psoriasis. In agreement with previously reported data,⁸ this analysis showed a significant increase in mast cells and CD4⁺ T cells in lesional skin than in nonlesional skin (see Fig E6, A-E, in this article's Online Repository available at www.jacionline.org). The frequency of mast cells in close apposition with CD4⁺ T cells was also increased (Fig E6, F). These cell-cell contacts exhibited morphologic features of immunologic synapses (Fig E6, C). Three-color IHC revealed that IL22⁺CD4⁺ T cells were enriched in psoriatic samples (Fig 4, A-C) and were found to form synaptic-like contacts with mast cells more frequently in lesional skin than in nonlesional skin (Fig 4, D). Moreover, when conjugated with mast cells, CD4⁺ T cells produced IL-22 more frequently than did their unconjugated counterparts (Fig 4, E). Similar results were obtained by analyzing skin biopsies from additional patients by using confocal laser scanning microscopy. This analysis

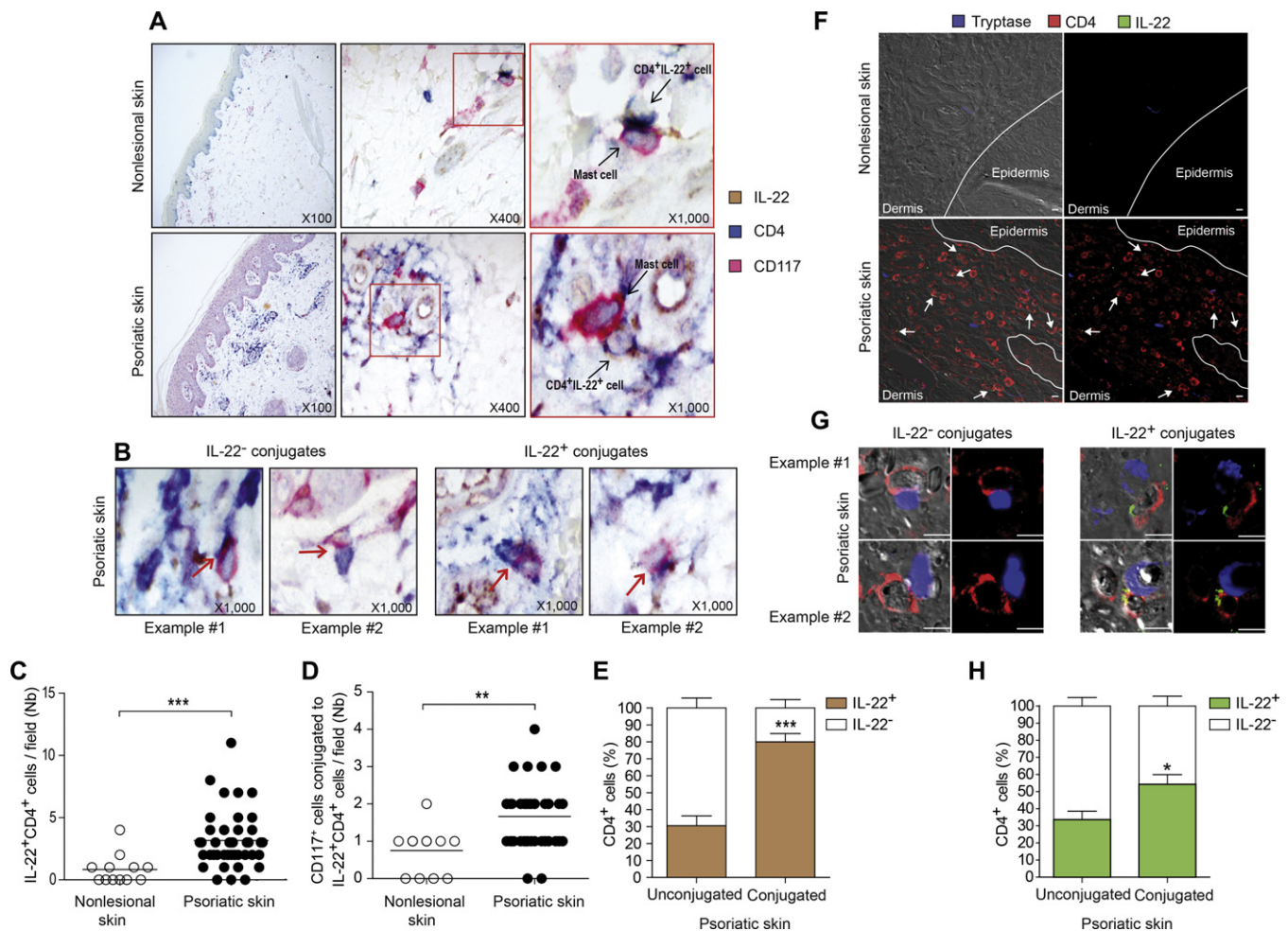


FIG 4. CD4⁺ T cells in close apposition with mast cells express IL-22 in psoriatic skin. Skin biopsies analyzed by using IHC (A-E) or confocal microscopy (F-H). Data from 2 nonlesional and 4 psoriatic skin biopsies (Fig 4, C-E). Each dot represents 1 analyzed field. Data from 3 psoriatic skin biopsies (Fig 4, H). Two-tailed, unpaired (Fig 4, C and D) and paired (Fig 4, E and H) *t* tests, ***P* < .01 and ****P* < .001.

showed that approximately 30% of unconjugated CD4⁺ T cells were IL-22⁺ in psoriatic skin while up to 60% of CD4⁺ T cells in contact with mast cells expressed IL-22 (Fig 4, F-H). An analysis performed by using the line-scan function of the MetaMorph software confirmed that cells scored as “conjugated” exhibited fluorescence proximity/overlap (see Fig E7 in this article’s Online Repository available at www.jacionline.org). In addition, in psoriatic skin, most of the IL-22⁺CD4⁺ T cells were IFN-γ⁺, indicating that a major IL-22/IFN-γ-producing CD4⁺ T-cell population is present *in vivo* (see Fig E8 in this article’s Online Repository available at www.jacionline.org).

Together these results support the findings that both T cells and mast cells are enriched in inflamed skin and reveal that T_H cells producing IL-22 and IFN-γ are strongly enriched in psoriatic skin, where they are mostly found in contact with mast cells.

DISCUSSION

In the present study, we addressed the question of whether hMCs might form cognate interactions with CD4⁺ T cells and have an impact on T_H cell biology. We report that IFN-γ-primed hMCs form immunologic synapses with previously activated

CD4⁺ T cells. Our results also reveal that T-cell/mast-cell cognate interactions fine-tune T_H cell responses by broadening the array of cytokines produced and by favoring the emergence of T_H22 and of IL-22/IFN-γ-producing T_H cells.

Similar to their mouse counterparts, hMCs acquired an APC phenotype after IFN-γ treatment.¹³ IFN-γ appears as a crucial signal in the commitment of hMCs toward cooperation with CD4⁺ T cells. In an inflamed tissue, several cells (eg, T_H1 cells, natural killer cells, and cytotoxic T lymphocytes) could be potential sources of IFN-γ required to induce mast-cell maturation toward an APC phenotype. It is tempting to speculate that in the course of a T_H1 immune response, T-cell-derived IFN-γ might promote the APC function of mast cells, which, in turn, might further amplify and diversify the immune response by serving as “tissue-localized” APC.

We used freshly isolated human memory CD4⁺ T cells as a model of *in vivo* antigen-experienced T cells. These cells recirculate throughout the body and are expected to encounter mast cells when they infiltrate tissues such as skin.^{18,25,26} These cells represent a mosaic of cells that have not been repeatedly stimulated and keep a certain level of plasticity in the panel of produced cytokines.²⁷⁻²⁹ Our results show that mast cells molded T_H responses

by broadening the panel of cytokine produced. IL-4 production was induced after interaction with mast cells but not with DC^{LPS}, which is compatible with the notion that mast cells are involved in T_H2-type responses under some pathologic conditions.¹² Interestingly, mast cells also induced T_H22, IL-22/IFN- γ , and IL-17/IL-22-producing T_H cells. This result was surprising and prompted us to further characterize these proinflammatory T_H cell subsets.

The induction of IL-22⁺ subsets was observed by Fujita et al when coculturing cutaneous DC (dermal DC or Langerhans cells) with allogenic whole peripheral T cells.³⁰ However, in this study, the main T_H-cell population generated was T_H22. In our study, hMC^{IFN- γ} mostly induced IL-22⁺/IFN- γ ⁺ T_H cells, indicating that mast cells might provide fate-determining signals less restricted than those of cutaneous DC.

What might be the origin of the observed IL-22⁺/IFN- γ ⁺ T_H population? It is reasonable to suggest that stimulation with mast cells might induce new IL-22⁺ T cells (mainly coexpressing IFN- γ) via a TNF- α /IL-6-dependent mechanism. This hypothesis is in agreement with the observation that treatment with anti-IL-6-R/TNF- α -blocking antibodies reduces the percentage of IL-22-producing cells in mast-cell-stimulated T cells and not in anti-CD3/CD28-stimulated T cells. Conversely, anti-CD3/CD28-coated beads might expand a preexisting IL-22⁺ memory T-cell population. In line with this hypothesis, we observed that the stimulation of freshly isolated memory T cells with anti-CD3/CD28-coated beads for 16 hours (followed by treatment with phorbol 12-myristate 13-acetate and ionomycin) resulted in 0.5% cells producing IL-22. Among these cells, 75% produced only IL-22 while 25% produced IL-22 plus IFN- γ (see Fig E9 in this article's Online Repository available at www.jacionline.org). Interestingly, this ratio was maintained after 6 days of culture with anti-CD3/CD28-coated beads (cf. Fig E9 and Fig 2). Further research is required to determine whether, in addition to IL-6 and TNF- α signaling, other signals provided at the immunologic synapse by mast cells contribute to IL-22 induction.

Our results, in line with previous reports,^{20,21} also show that most of the IL-22⁺CD4⁺ T cells induced under our experimental conditions expressed CCR4 and CCR6 as well as the AHR transcription factor, which are typical markers of T_H22 cells.^{20,21} CCR4 binds CCL17 and CCL22, both produced by keratinocytes in response to inflammatory signals.³¹ CCR6 binds CCL20, which is produced in the skin in response to inflammation and also by mast cells in response to *Pseudomonas aeruginosa*.³² CCR4 and CCR6 could therefore guide recirculating memory T cells into the skin or mucosa to favor their interaction with keratinocytes or mast cells and might help IL-22⁺ T_H cells to remain in the inflamed skin.²⁶

Multiple lines of evidence support a functional relationship between mast cells and T cells in several inflammatory disorders.³³ In the course of an immune response in the skin or mucosa, mast cells could thus be instrumental in providing IL-22 differentiating signals. We furnish here the first evidence in favor of the hypothesis that a functional T-cell/mast-cell interaction leading to IL-22 production could occur in human inflamed skin. Our results show a rich infiltrate of CD4⁺ T cells in close contact with mast cells and producing IL-22 in psoriatic skin. In addition, most of these IL-22⁺CD4⁺ T cells were IFN- γ ⁺. IL-22 and IFN- γ are known to play a central role in psoriasis pathogenesis.^{22,34,35} Our results extend this knowledge and suggest that

immunologic synapses formed between T cells and mast cells contribute to the generation of IL-22⁺/IFN- γ ⁺ T_H cells in psoriatic skin and, in turn, to disease progression. IL-22 and IFN- γ are overexpressed in other T_H-cell-related inflammatory skin disorders such as contact dermatitis or atopic eczema.²³ Further research is required to define whether mast cells might also contribute to drive T_H cells toward IL-22/IFN- γ production in these pathologies. It would also be interesting to define whether IL-4, a cytokine induced *in vitro* by mast cells (Fig 2), might be detected in mast-cell-conjugated T_H cells in this skin disorder.

In summary, our results shed new light on the functional outcome of T_H-cell/mast-cell cognate interactions by showing that mast cells can shape the inflammatory potential of human memory T lymphocytes. Under physiologic conditions, these interactions might serve to diversify biological responses of memory T cells entering tissues as well as to shift the balance of local responses toward protective inflammatory reactions. According to this viewpoint, the IFN- γ produced by incoming T_H cells would enhance the antigen-presenting potential of mast cells, which, in turn, by presenting antigenic determinants to memory T cells, would amplify IFN- γ - and IL-22-based responses. Under uncontrolled inflammatory conditions, such as in psoriatic skin, the presence of an abundant immune cell infiltrate might exacerbate the IFN- γ /IL-22-based inflammatory loop, turning T-cell/mast-cell interactions into "dangerous liaisons" for tissue integrity.

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Key messages

- Cognate interactions between human mast cells and memory CD4⁺ T cells led to the emergence of IL-22- and IL-22/IFN- γ -producing T_H cells.
- The major part of CD4⁺ T cells conjugated with mast cells in lesional psoriatic skin biopsy specimens expressed IL-22 alone or in combination with IFN- γ .
- These data reveal a novel functional trait of mast cells by showing that they can play the role of APCs to shape the inflammatory potential of human T_H lymphocytes.

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METHODS

Antibodies

Anti-CD4-FITC (RPA-T4), anti-CD117-PEcy5 (YB5.B8), anti-CD14-PE (MφP9), anti-CD45RO-PE (UCHL1), anti-CD11c-PEcy5 (B-ly6), anti-IFN- γ -FITC (B27), anti-IL-4-APC (MP4-25D2), anti-IL-17A-alexa647 (SCPL1362), anti-HLA-DR-PEcy5 (L243), anti-CD54 (HA58), anti-CD80 (L307.4), anti-CCR6-PerCP-Cy5.5 (11A9), anti-CCR4-Biotin (1G1), anti-CCR10-APC (6588-5), and isotype controls were from Becton, Dickinson and Company; anti-IL-22-PE (142928) was from R&D Systems; anti-mast cell tryptase (FL-275), anti-Ah receptor (H-211), anti-pTyr (PY99), anti-CD3e (SH-T1), and anti-IL-22 (E-17) were from Santa Cruz Biotechnology; anti-Fc ϵ RI-FITC (AER-37) and anti-mouse secondary mAbs were from e-Bioscience; anti-CD4 (1F6) was from DAKO; streptavidin-alexa488, anti-rabbit-alexa647, anti-goat-alexa488, and anti-mouse-alexa555 were from Invitrogen.

Cell purification and sorting

PBMCs were obtained from buffy coats (Etablissement Français du Sang). CD133⁺ precursor cells were isolated from PBMCs by using CD133-specific microbeads (130-050-801, Miltenyi Biotec). CD4⁺ T cells were isolated from PBMCs by using a CD4⁺ T-Cell Isolation Kit II (130-091-155, Miltenyi Biotec); then, V β 2⁺ cells were sorted by using anti-V β 2-specific mAbs (MPB2D5, Beckman Coulter, Fullerton, Calif) and goat antimouse IgG microbeads (130-048-402, Miltenyi Biotec). Naive CD4⁺ T cells were isolated from PBMCs by using a naive CD4⁺ T-Cell Isolation Kit II (130-094-131, Miltenyi Biotec). CLA⁺CD4⁺ T cells were isolated from PBMCs by using an Anti-CLA MicroBead Kit (130-092-464, Miltenyi Biotec), with a purity of at least 95%. Memory CD4⁺ T cells were isolated from PBMCs (immunomagnetic isolation of untouched human memory CD45RA⁺CD45RO⁺CD4⁺ T cells) by using a human memory CD4⁺ T-cell enrichment kit (19157, stem cell technology), with a purity of at least 96%.

Phenotypical and functional characterization of hMCs

hMCs were characterized by using histochemistry: cell suspensions were cytocentrifuged onto glass slides, air dried, and stained with toluidine blue. hMCs were characterized by using IHC: cells were probed with anti-tryptase pAbs as described in the Confocal Microscopy Analysis of T-Cell/Mast-Cell Conjugates section and revealed with horseradish peroxidase-labeled secondary mAbs (Invitrogen) and stained by using an AEC Staining Kit (AEC-101, Sigma). Mast cell degranulation was determined by measuring the release of β -hexosaminidase. hMCs were sensitized with human IgE for 16 hours; the cells were then washed and distributed in 96-well flat-bottom plates at a density of 1×10^5 cells in 50 μ L of Tyrode's buffer. The cells were adapted to 37°C for 20 minutes and then treated with prewarmed 50 μ L of the indicated stimuli (substance P, compound 48/80 [Sigma], C5a [R&D Systems, Minneapolis, Minn], or antihuman IgE [Santa Cruz Biotechnology]) diluted in Tyrode's buffer for 45 minutes at 37°C. The release of β -hexosaminidase

in the supernatants was measured by means of enzymatic colorimetric analysis with *p*-nitrophenyl *N*-acetyl- β -D-glucosamine (Sigma). Phenotypic and functional analyses were performed on hMCs after 8 weeks of culture.

DC culture and priming

CD14⁺ cells were seeded at 2×10^5 cells/mL in a complete RPMI 1640 medium (10% heat-inactivated FCS, GlutaMAX-I, sodium pyruvate, 2-mercaptoethanol, cyprofloxacin [10 μ g/mL, Sigma-Aldrich]) supplemented with IL-4 (100 IU/mL) and GM-CSF (750 IU/mL) (both from R&D Systems) and cultured for 6 days. DCs were primed during 16 hours with LPS (500 ng/mL, Sigma-Aldrich) and referred to as DC^{LPS}.

Intracellular cytokine staining and enzyme-linked immunosorbent assay

After 6 days of coculture, intracellular cytokines were analyzed on CD4⁺ T cells after 5-hour restimulation with phorbol 12-myristate 13-acetate (50 ng/mL, Sigma) and ionomycin (1 μ g/mL, Sigma) in the presence of GolgiStop (BD Pharmingen). Cells were fixed, permeabilized (0.3% saponin in PBS containing 1% BSA, Sigma-Aldrich), and stained with antibodies directed against IFN- γ , IL-4, IL-17A, and IL-22. Flow cytometric data were acquired on a BD Biosciences FACScalibur or BD LSR-II cytometer and were analyzed by using FlowJo software (Tree Star, Inc, Ashland, Ore).

Cytokine production was measured with sandwich ELISA by using commercial matched-pair antibodies (IFN- γ : Biosource; IL-22: Peprotech), streptavidin-HRP (SouthernBiotech), and TMB (Sigma) according to the manufacturer's instructions. IL-4 and IL-17 production was measured by using CBA flex detection kits (BD Pharmingen).

Confocal microscopy analysis of T-cell/mast cell conjugates

A total of 1×10^5 CD4⁺V β 2⁺ T cells were plated with 2×10^5 mast cells either unpulsed or pulsed with 50 ng/mL of TSST-1 in 96-well U-bottom plates and spun down at 500 rpm for 30 seconds to allow conjugate formation and incubated for 5 minutes at 37°C. Cells were transferred to poly-L-lysine-coated slides; then, they were fixed, permeabilized, and stained with anti-CD3e and anti-pTyr antibodies followed by secondary antibodies and 2 μ g/mL of avidin-sulforhodamine 101 (a highly cationic glycoprotein that selectively stains mast cell granules,^{E1} Sigma). Samples were examined with a Zeiss LSM 710 confocal microscope, as described previously.^{E2} Unprocessed images were analyzed by using region measurement and line-scan functions of the MetaMorph software (Universal Imaging, Downingtown, Pa), as described previously.^{E2}

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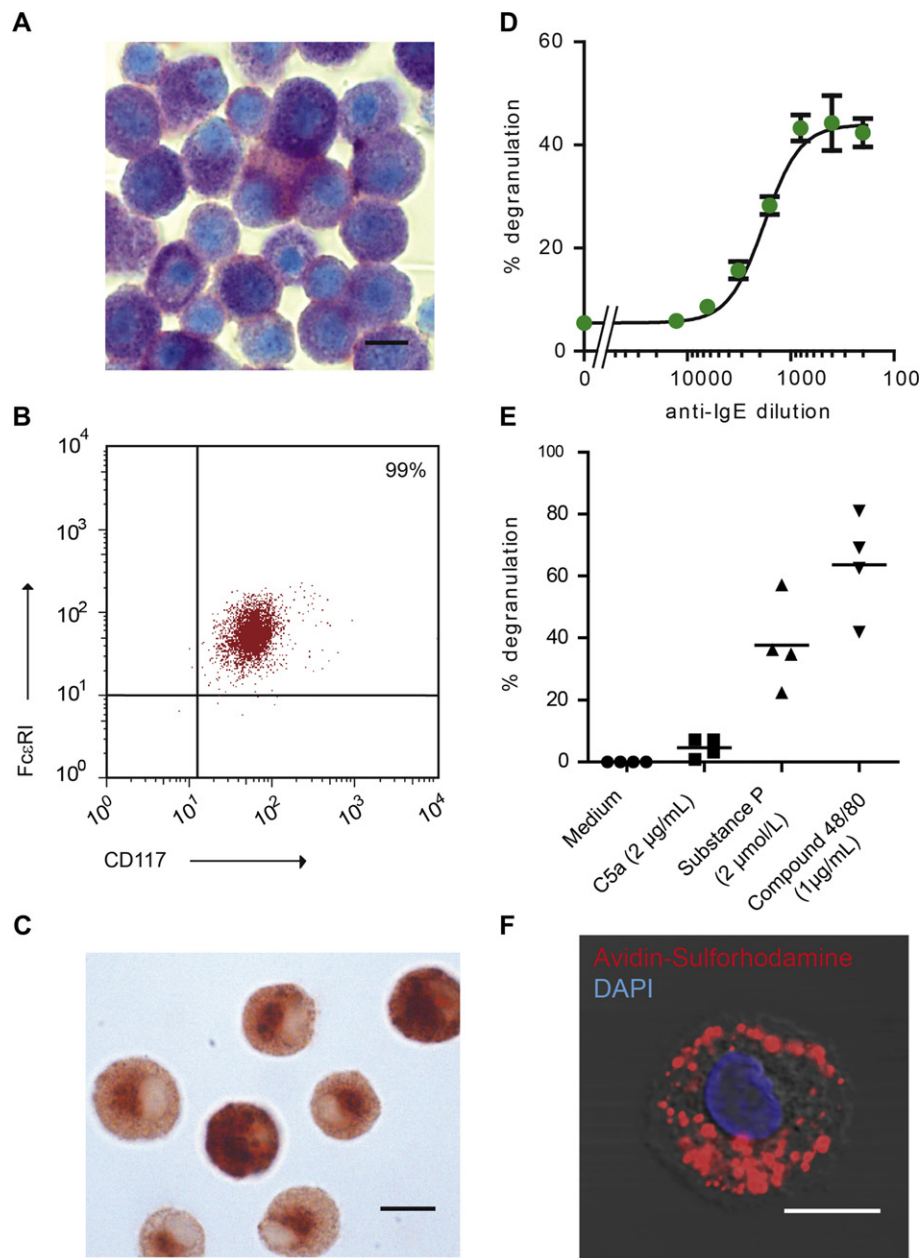


FIG E1. Phenotypic and functional characterization of hMCs. **A**, Histochemical staining of hMCs with toluidine blue. **B**, Flow cytometry analysis of CD117 and FcεRI expression on the hMC surface. **C**, Mast cell tryptase staining by immunocytochemistry. Bar = 10 μm. **D**, FcεRI-dependent β-hexosaminidase release. **E**, Secretagogue or C5a-induced β-hexosaminidase release. **F**, Confocal visualization of a representative hMC. Avidin-sulforhodamine 101 (red) and 4'-6-diamidino-2-phenylindole (DAPI; blue) are shown. Data are from 1 of 5 representative experiments.

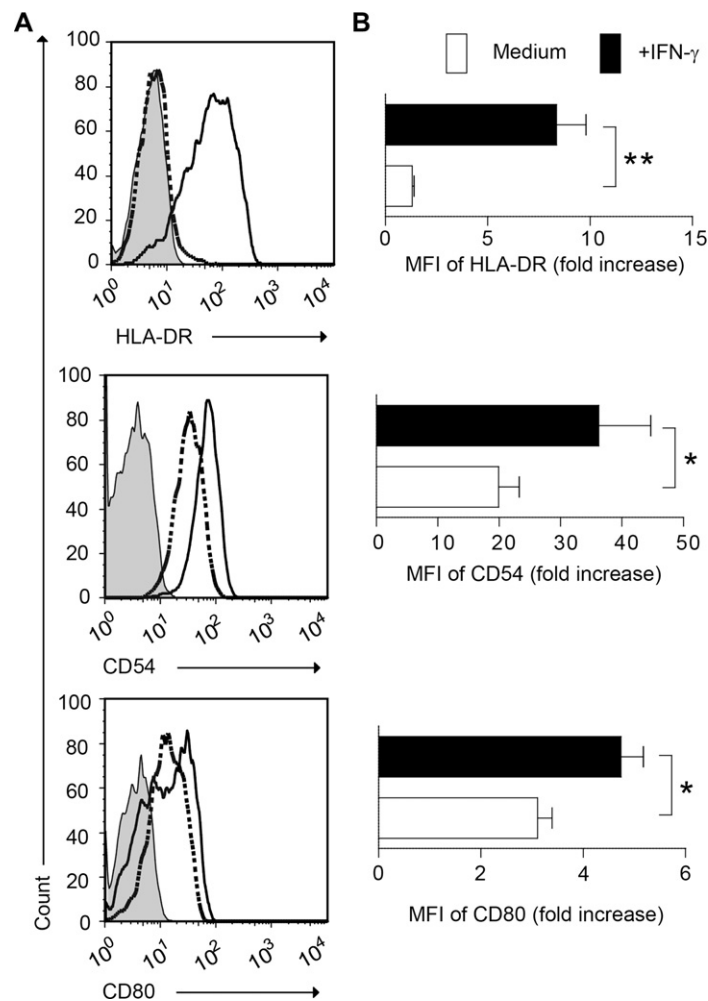


FIG E2. IFN- γ -primed hMCs express HLA-DR and costimulatory molecules. Flow cytometry analysis of the expression of surface HLA-DR, CD54, and CD80 after 72-hour incubation with IFN- γ (50 ng/mL). **A**, Representative histograms are depicted in the left panel (black lines, IFN- γ treatment; dotted lines, untreated; shaded areas, isotype-matched control antibodies). **B**, Fold increase in geometric mean fluorescence intensity (MFI) over isotype control is shown in the right panel. Data are from 6 independent experiments. Two-tailed, unpaired t test, * $P < .05$ and ** $P < .01$.

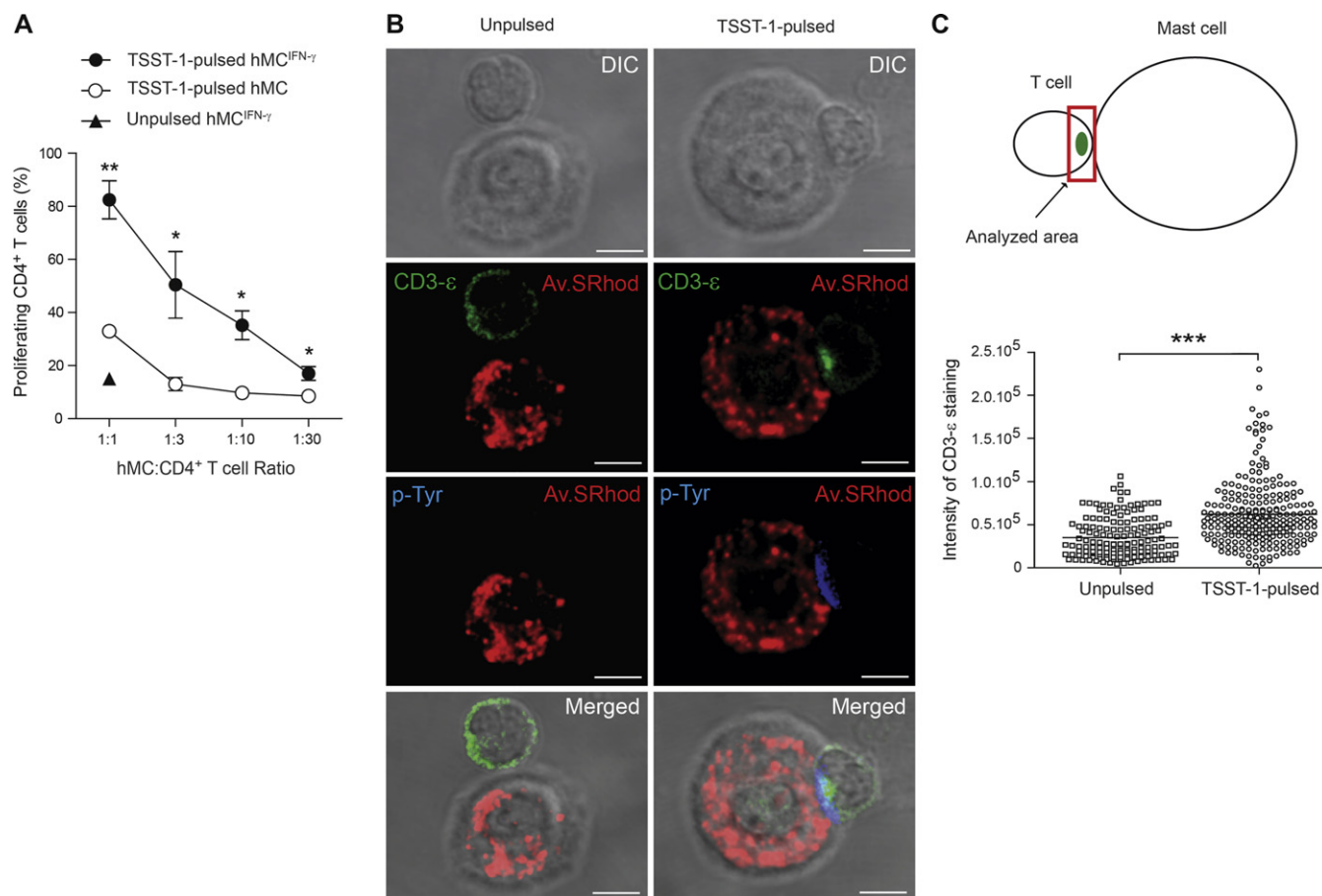


FIG E3. hMC^{IFN-γ} induce functional responses in T_H cells. **A**, Proliferation of CD4⁺Vβ2⁺ T cells cocultured with hMCs or hMC^{IFN-γ}. **B**, hMC^{IFN-γ}/CD4⁺Vβ2⁺ T-cell conjugates were stained with avidin-sulforhodamine 101 (red), CD3ε (green), and pTyr (blue). **C**, Quantification of CD3ε staining at the contact site. Each point represents 1 conjugate. Mean ± SEM of 3 independent experiments. Two-tailed, unpaired *t* test **P* < .05, ***P* < .01, and ****P* < .001.

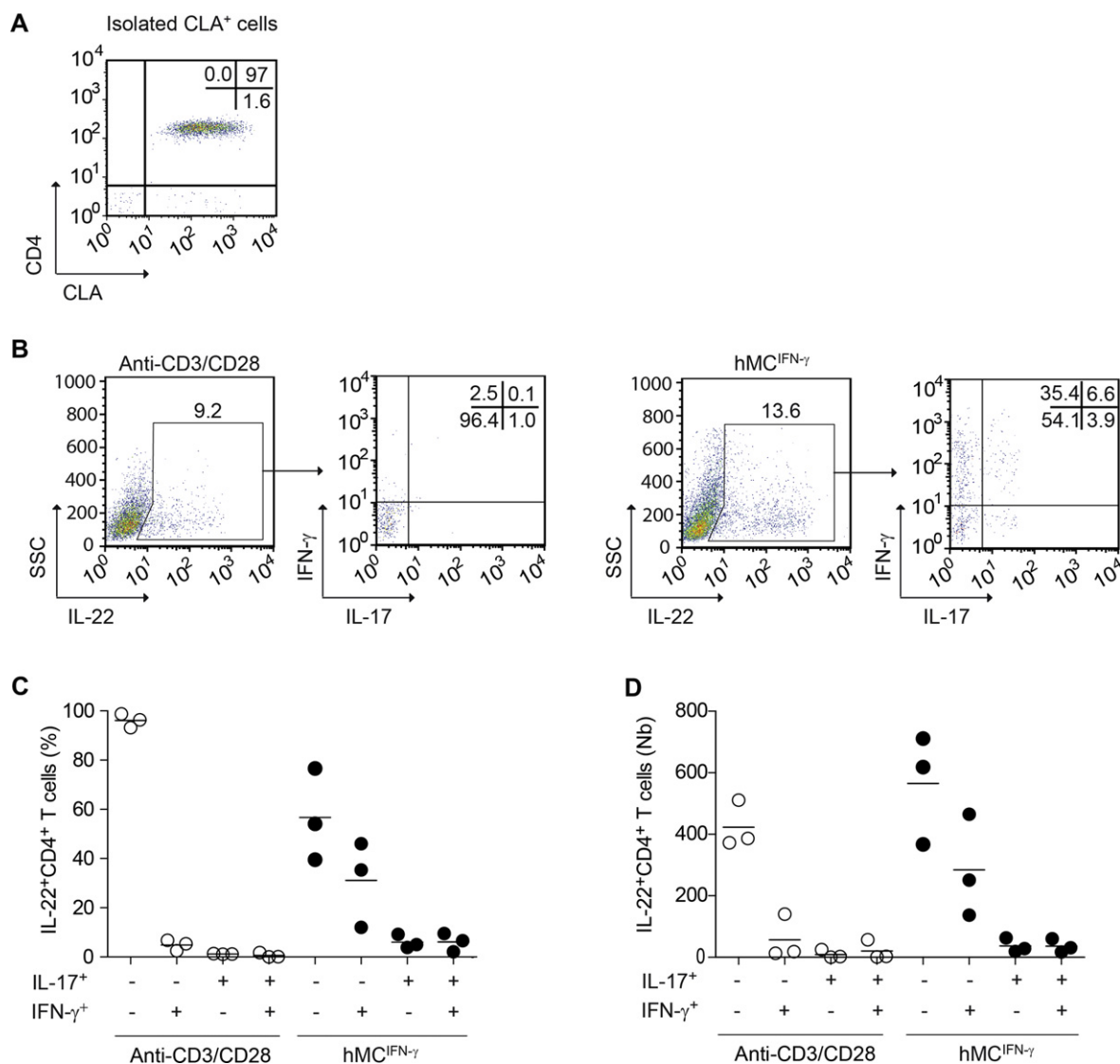


FIG E4. hMC^{IFN-γ} induce CLA⁺CD4⁺ T cells to produce IL-22 alone or in combination with IFN-γ. Skin homing CLA⁺CD4⁺ T cells were stimulated as described in Fig 3. **A**, Purity of CLA⁺CD4⁺ T cells after isolation from PBMCs. **B**, IL-17- and IFN-γ-producing cells among IL-22⁺CD4⁺ T cells. **C** and **D**, Frequency (Fig E4, C) and numbers (Fig E4, D) of IL-22⁺CD4⁺ T cells expressing IL-17, IFN-γ, or both. Horizontal bars indicate mean. Each symbol represents 1 experiment with 1 donor.

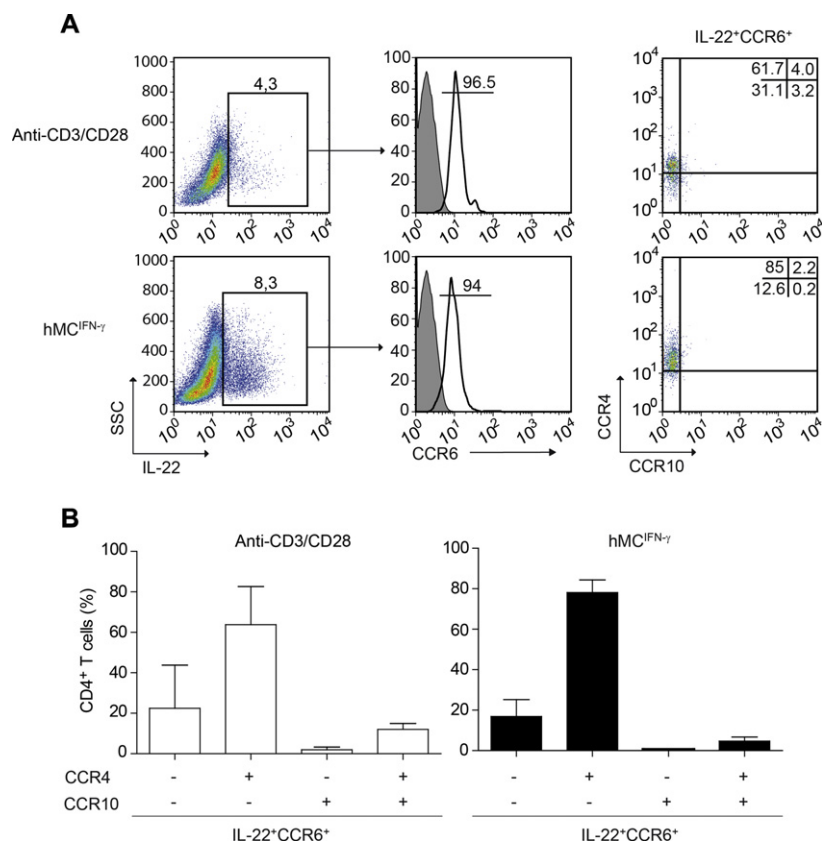


FIG E5. Most IL-22⁺ T_H cells express CCR4 and CCR6. Freshly isolated memory CD4⁺CD45RO⁺CD45RA⁻ T cells were cocultured for 6 days with anti-CD3/CD28-coated beads or with SAg-loaded (50 ng/mL) hMCIIFN- γ and stimulated for 5 hours with phorbol 12-myristate 13-acetate and ionomycin. **A**, Representative dot plots of the flow cytometry analysis of CCR6 expression on IL-22⁺ T cells and CCR4/CCR10 expression on IL-22⁺CCR6⁺CD4⁺ T cells. **B**, Frequency of IL-22⁺CCR6⁺CD4⁺ T cells expressing CCR4, CCR10, or both. Data represent mean $\pm$ SEM of 3 independent experiments using 3 different donors.

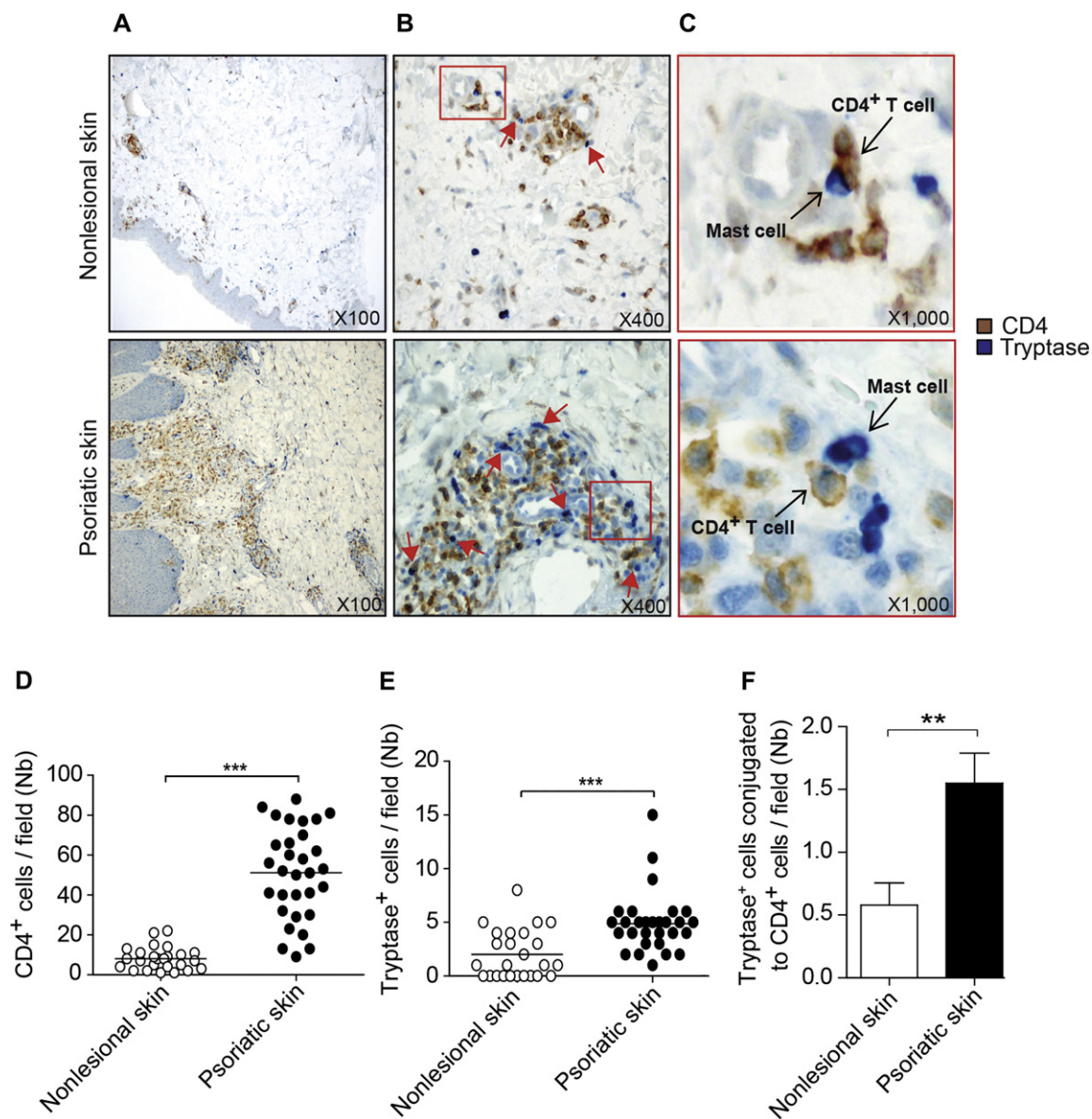


FIG E6. Mast cells and CD4⁺ T cells establish synaptic-like contacts in psoriatic skin. **A**, Representative sections from nonlesional and psoriatic skin, showing CD4⁺ (brown) and tryptase⁺ (blue) cells as detected by IHC. **B** and **C**, Examples of CD4⁺ cell/tryptase⁺ cell interactions (red arrows). **D-F**, CD4⁺ and tryptase⁺ cells per field and CD4⁺/tryptase⁺ conjugated cell number per field were scored from psoriatic (5 patients) or nonlesional (4 patients) skin samples. Several fields were analyzed at $\times 400$ magnification. Each dot in (Fig E6, D) and (Fig E6, E) represents data scored in 1 field, $**P < .01$ and $***P < .001$.

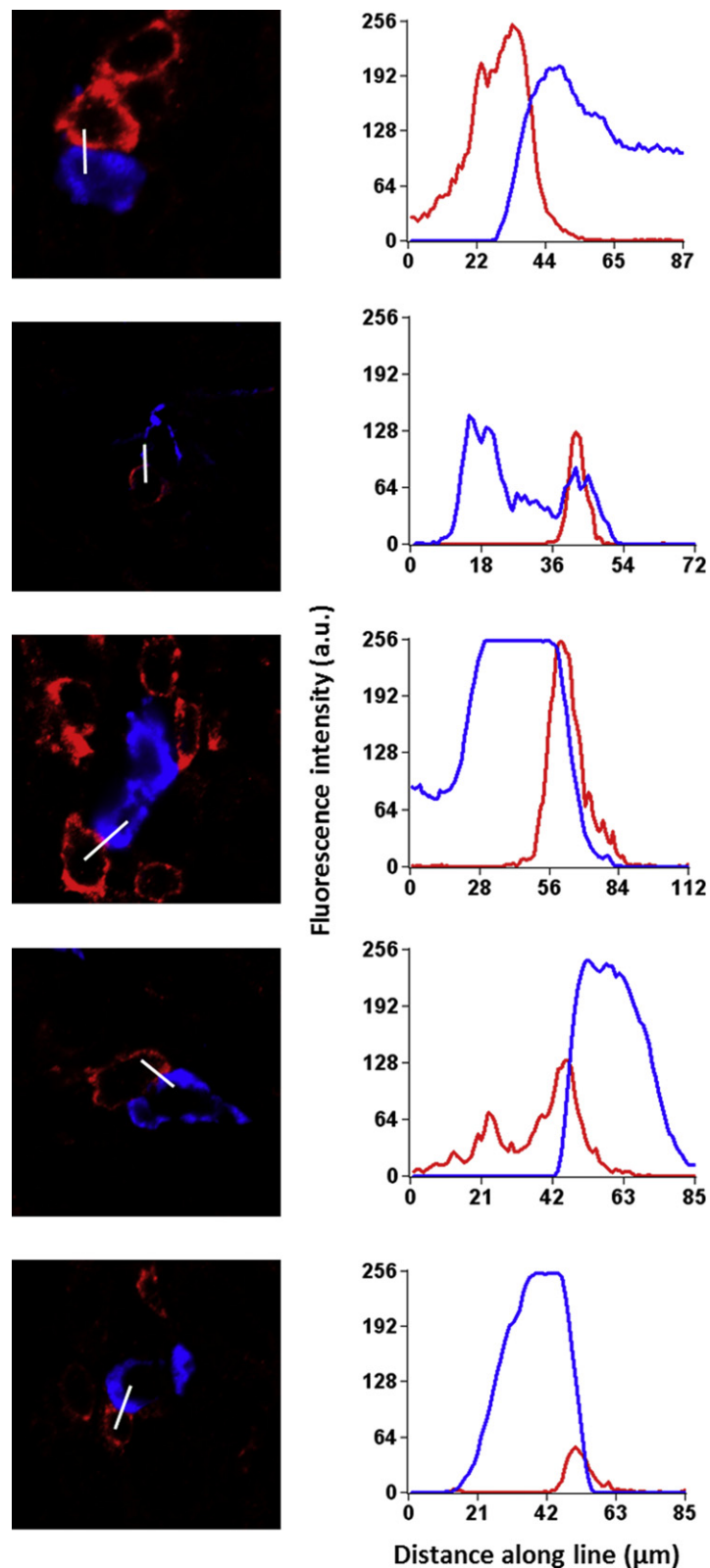


FIG E7. Synaptic-like contacts analysis. The 5 images in the *left panels* show examples of T cells (stained for CD4 in *red*) and mast cells (stained for tryptase in *blue*) scored as “conjugated” in the analysis depicted in Fig 5. The *white line* drawn across the cell-cell contact indicates the position of the line-scan analysis presented in the *right panels*.

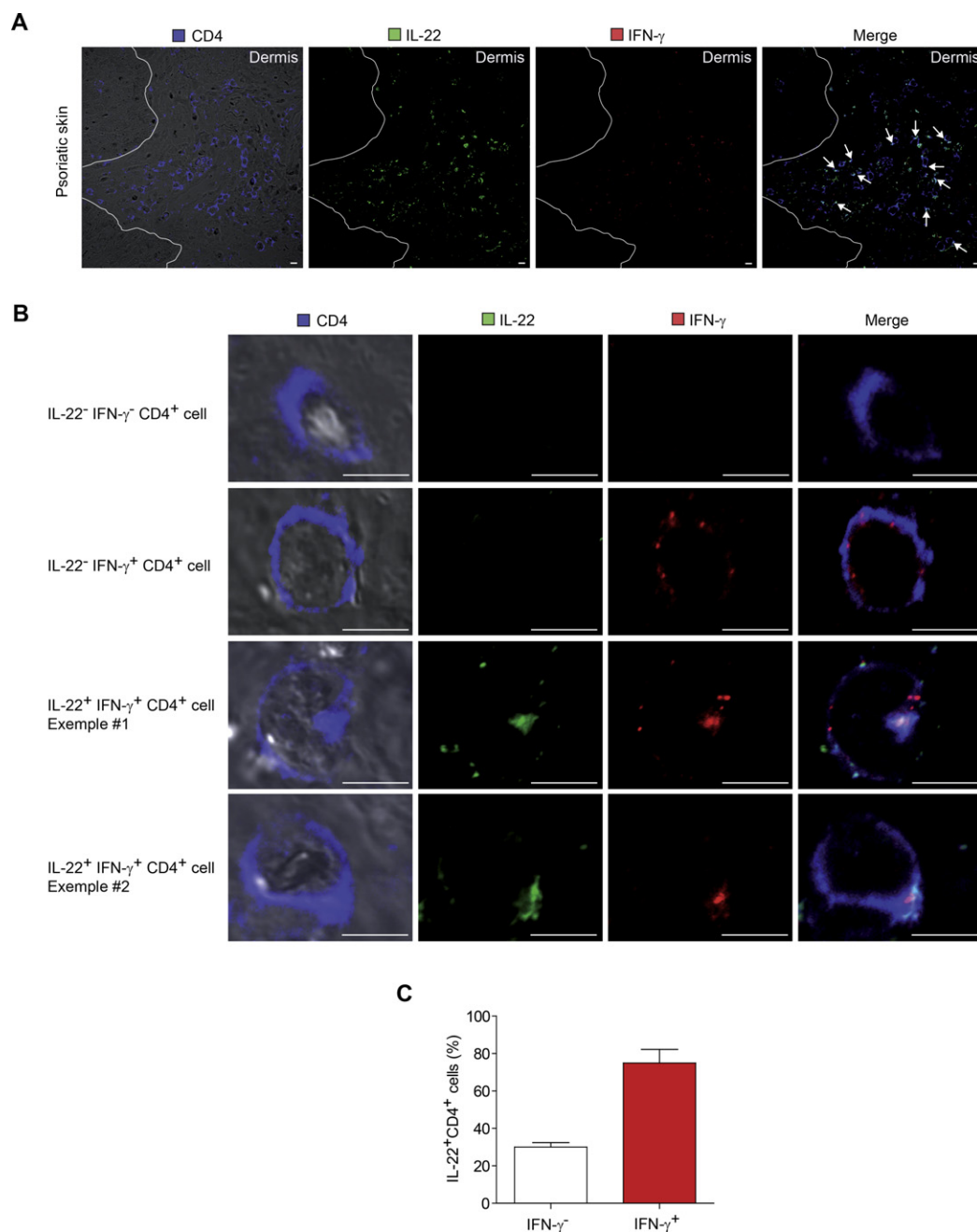


FIG E8. Most of the IL-22-producing CD4⁺ T cells produce concomitantly IFN- γ in psoriatic skin. **A**, Sections from the psoriatic skin sample showing CD4⁺ (blue), IFN- γ ⁺ (red), and IL-22⁺ (green) cells. Bar = 5 μ m. White arrows indicate IFN- γ ⁺IL-22⁺CD4⁺ T cells. **B**, Four examples of CD4⁺ T cells positive or not for IFN- γ and/or IL-22 staining. Bar = 5 μ m. **C**, Percentage of IL-22⁺CD4⁺ T cells producing IFN- γ (red bar) or not (white bar) in psoriatic skin. Mean $\pm$ SEM are from 2 patients with psoriasis.

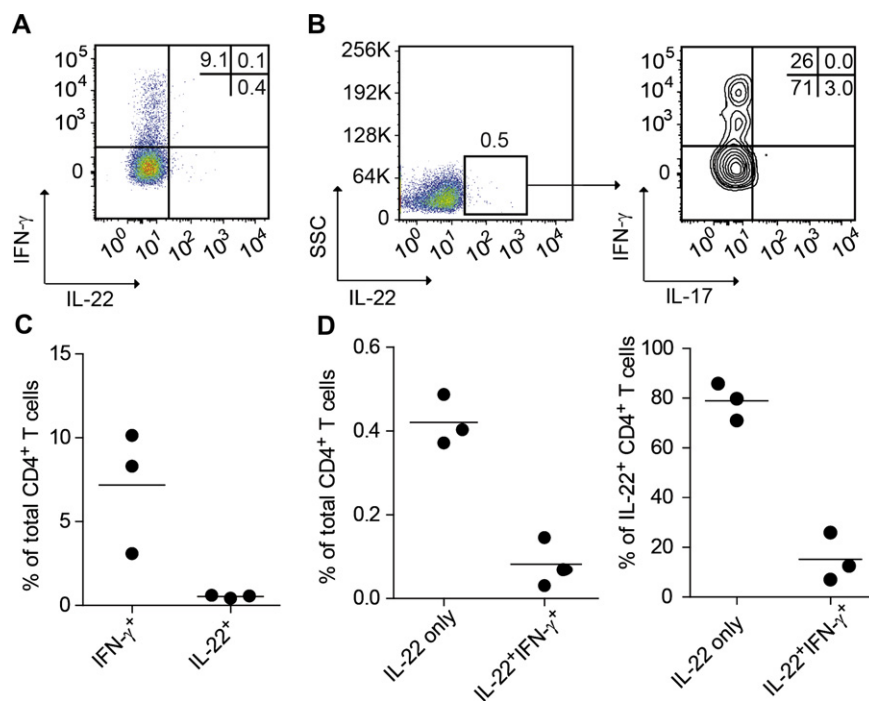


FIG E9. Cytokine production analysis by CD4⁺ T cells after overnight stimulation with anti-CD3/CD28-coated beads. Freshly isolated memory CD4⁺CD45RO⁺CD45RA⁻ T cells were cocultured for 16 hours with anti-CD3/CD28-coated beads and stimulated for 5 hours with phorbol 12-myristate 13-acetate and ionomycin before flow cytometry analysis. **A** and **B**, Representative *dot plots*. **C** and **D**, Data are from 3 donors; *bars* represent the mean. Each *symbol* represents 1 donor.

VIDEO E1. Cognate interactions with hMC^{IFN- γ} induce sustained $[Ca^{2+}]_i$ increase in CD4⁺V β 2⁺ T cells: 2x10⁵ hMC^{IFN- γ} were loaded or not with 50ng/mL TSST-1, stained with CMTMR (*red*) and plated with 1x10⁵ CD4⁺V β 2⁺ T cells loaded with Fluo4 (*green*) in microchambers (Lab-Tek Coverglass System; Nunc) previously coated with poly-D-lysine hydrobromide (50 μ g/mL; Sigma) in complete RPMI 1640. After addition of T cells, fluorescence was acquired every 5 seconds using Zeiss LSM 710 confocal microscope at 37°C, 5% CO₂. Image sequences of the time-lapse recording were processed using the Zen software.