

Letter to the Editor

Chemical toxicity to keratinocytes triggers dendritic cell activation via an IL-1 α path

To the Editor:

Keratinocytes, which make up the bulk of the skin epidermis, release a range of immunomodulatory factors, including cytokines and chemokines, which play a key role in regulating the local adaptive immune response.¹ However, toxicity of keratinocytes is found in several skin diseases. Environmental influences such as chemicals, ultraviolet light, mechanical insult, or extreme temperatures can cause necrosis of keratinocytes, leading to failure of local homeostasis, breaching of the skin barrier, and the potential for infection. Keratinocytes produce the IL-1 α

precursor constitutively and store it intracellularly. The role of this intracellular IL-1 α remains unclear.² We tested the hypothesis that dendritic cells (DCs), the sentinels of adaptive immunity, can respond directly to chemically induced keratinocyte damage via IL-1 α released from the dying cells.

The response of human DCs to IL-1 α has not previously been studied in detail, although IL-1 α has been shown to regulate mouse Langerhans cell motility.³ Monocyte-derived DCs (prepared as described in Le Bert et al.⁴) cultured in the presence of recombinant IL-1 α (Peprotech, London, United Kingdom) for 20 hours were analyzed for cell surface phenotype by flow cytometry (as described in Le Bert et al.,⁴ antibodies from BD

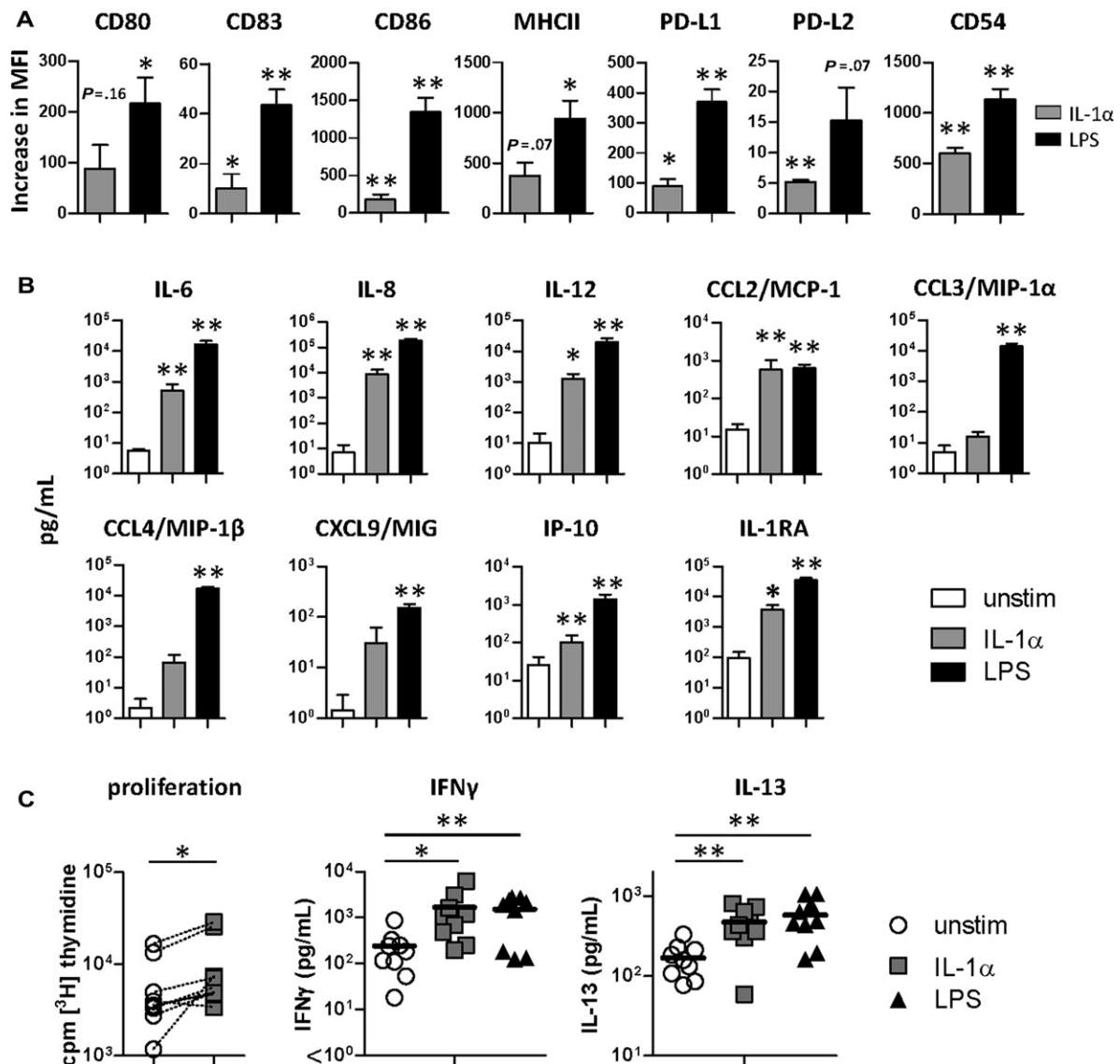
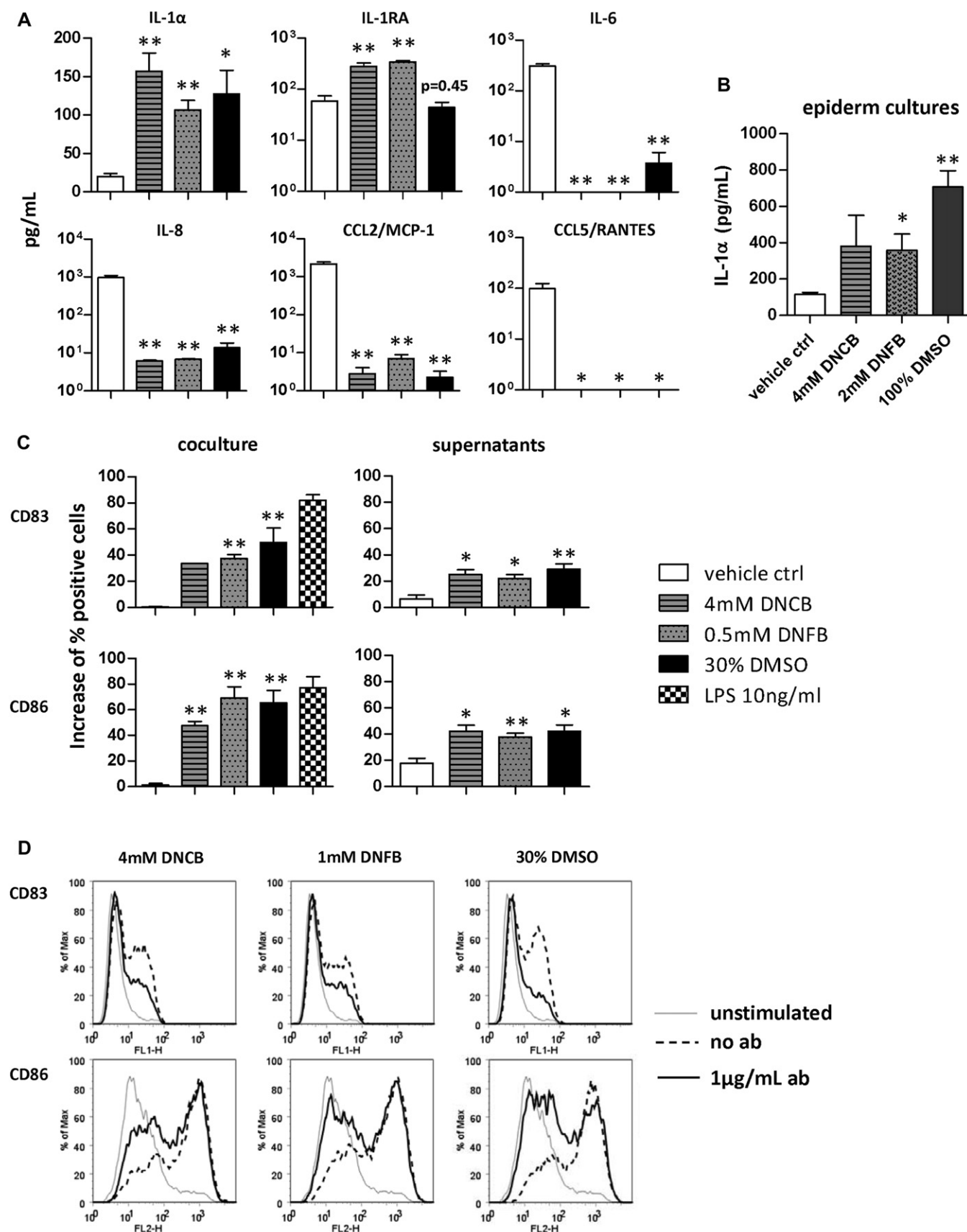


FIG 1. The response of DCs to IL-1 α . DCs were stimulated with IL-1 α or LPS for 20 hours. **A**, Increase in median fluorescent intensity relative to unstimulated cells (mean $\pm$ SEM, $n \geq 4$). Representative plots shown in Fig E2. **B**, Supernatant cytokine levels (mean $\pm$ SEM, $n = 4$). **C**, Allogeneic T-cell proliferation and cytokine production. Each point is an independent experiment, and horizontal line shows the mean. * $P < .05$, ** $P < .01$ (paired Student t test). unstim, Unstimulated.



Biosciences, Oxford, United Kingdom). IL-1 α (10 ng/mL saturating concentration; for dose-response, see Fig E1 in this article's Online Repository at www.jacionline.org) led to an increased expression of the costimulatory molecules CD54, CD80, CD83, and CD86, to MHC class II, and the inhibitory surface receptors PD-L1 and PD-L2 on the DC surface (Fig 1, A; see Fig E2 in this article's Online Repository at www.jacionline.org). This phenotype is very characteristic of classical DC activation in response to pathogen-associated molecular patterns, although the increase was less pronounced than with a saturating dose of the prototypical Toll-like receptor agonist LPS (100 ng/mL; *Escherichia coli* O111:B4, Invivogen, Nottingham, United Kingdom). Luminex analysis (Invitrogen kit, measured on Luminex 200IS platform, Invitrogen, Paisley, United Kingdom) of IL-1 α -stimulated DC supernatants 24 hours after stimulation (Fig 1, B) also detected a number of proinflammatory cytokines and chemokines including IL-6, IL-8, IL-12, CCL2/MCP-1, CCL3/MIP-1 α , CCL4/MIP-1 β , CXCL9/MIG, IP-10, and IL-1 receptor antagonist (IL-1RA).

IL-1 α -stimulated DCs also showed an increased ability to stimulate allogeneic T-cell responses (Fig 1, C). DCs (1000) were cocultured with CD4⁺ allogeneic T cells (10⁵), and proliferation (³H thymidine incorporation; day 3) and cytokine release (ELISA, eBioscience, Hatfield, United Kingdom; day 5) were measured. IL-1 α -stimulated DCs induced increased T-cell proliferation compared with unstimulated DCs and increased the release of IFN- γ and IL-13 in the DC-T-cell cocultures. IL-1 α did not alter IL-17 levels (not shown).

We next investigated the ability of keratinocytes to activate DCs. The transformed cell line HaCaT is widely used as a model of human keratinocytes. The HaCaT cells were exposed for 1 hour to 2 skin sensitizers (2,4-dinitrochlorobenzene [DNCB] and 1-fluoro-2,4-dinitrobenzene [DNFB] from Sigma-Aldrich [Gillingham, United Kingdom], stock solutions in dimethyl sulfoxide [DMSO] from Acros Organics [Loughborough, United Kingdom], and diluted in PBS) or DMSO (a skin irritant). The chemicals were washed off, and the HaCaT cells were cocultured with DCs for further 20 hours. Annexin V and propidium iodide staining after chemical treatment and a 1-hour culture period showed that most cells went into necrosis (see Fig E3 in this article's Online Repository at www.jacionline.org). Luminex analysis of HaCaT supernatants showed that viable HaCaTs (vehicle control group) constitutively produced low levels of CCL5 and IP-10 and high levels of IL-6, IL-8, and CCL2 (Fig 2, A). However, production was abolished by chemical treatment. In contrast, the release of IL-1 α (eBioscience's ELISA kit, Hatfield, United Kingdom) and IL-1 receptor antagonist was increased by chemical exposure. In order to confirm these findings in a more physiologically relevant model, we used Epiderm skin models (MatTek, Ashland, Ore), 3D skinlike tissue structures derived from primary human keratinocytes. By using this model, we demonstrated that primary keratinocytes also release IL-1 α when exposed to cytotoxic concentrations of chemicals (Fig 2, B). The results support a model in which

IL-1 α and IL-1 receptor antagonist are stored in keratinocytes and released during cell necrosis.

In order to explore the role of keratinocyte IL-1 α in stimulating DCs, the HaCaT cells were treated with chemicals as mentioned above and cocultured with DCs in a ratio of 1 DC:50 HaCaT cells. Exposure of DCs to chemically treated HaCaT cells led to the activation of DCs measured by CD83 and CD86 upregulation (Fig 2, C). The upregulation of CD86 was comparable to the levels after LPS stimulation. Next, we wanted to assess whether the supernatant of chemically killed HaCaT cells alone is sufficient to induce DC activation. Therefore, the HaCaT cells were treated with different chemicals for 1 hour and afterward cultured for 20 hours. The supernatants were used to activate DCs for 20 hours. Fig 2, C, shows that the supernatant is sufficient to induce DC maturation measured by CD83 and CD86, although to a lesser extent than exposure to treated cells. No difference in terms of DC activation was observed between sensitizers and irritants in either the coculture or the supernatant experiments.

Having demonstrated that the HaCaT cells release IL-1 α during chemically induced cell death and also induce DC maturation and cytokine release, we wanted to establish whether the IL-1 α was directly required for the DC activation in this model. The HaCaT cells were treated with chemicals for 1 hour and, after washing in PBS, cultured for 20 hours. The supernatants were incubated at 37°C for 30 minutes with an anti-IL-1 α -blocking antibody (R&D Systems, Abingdon, United Kingdom) or a goat IgG control before adding in a 1:2 dilution to the DCs. Supernatants from chemically treated cells induced CD83 and CD86 upregulation on the DCs. Blocking of IL-1 α significantly reduced the amount of CD83 and CD86 expression, showing that IL-1 α released by dying keratinocytes contributes to DC activation in this model (Fig 2, D). Blocking was not complete, suggesting the presence of other danger signals in the supernatants.

We propose that the regulation of DC maturation, and the consequent engagement of adaptive immunity, can occur via at least 3 complementary routes. Skin DCs (both Langerhans cells and dermal DCs) can respond directly to interaction with an infectious agent, as has been documented extensively for herpes simplex virus, for example, Pollara et al.⁵ and Reske et al.⁶ Keratinocytes themselves can actively play a role in modulating local immunological responses, releasing inflammatory and chemoattractant cytokines and chemokines in response to infection.^{7,8} However, both these routes are compromised when the magnitude of the infectious, chemical or physical insult is so great as to result in extensive cytotoxicity of keratinocytes and DCs. Under these circumstances, the third route elaborated in this study may become important. Dying keratinocytes in the immediate area of the insult will release IL-1 α and other soluble molecules that will diffuse away from the focus of the injury to adjacent sites where they will activate DCs to produce cytokines/chemokines that will attract other cellular components of immunity. Activated DCs will subsequently migrate to lymphoid tissue carrying antigens acquired in the skin and induce an adaptive immune response that will provide subsequent protection from infection.

FIG 2. Chemical injury activates DCs via the release of IL-1 α from keratinocytes. Cytokine levels in (A) supernatants of the HaCaT cells ($n \geq 4$, chemicals and concentrations as in legend to C) or (B) EpiDerm skin cultures ($n = 3$). C, Phenotype (median fluorescent intensity relative to unstimulated cells [mean $\pm$ SEM, $n = 2-4$]) of DCs cocultured with chemically treated HaCaT cells or HaCaT supernatant. D, As for C but anti-IL-1 α or control antibody was added to supernatants before addition to DCs. One of 3 experiments. * $P < .05$, ** $P < .01$ (paired Student t test).

In certain circumstances, the same pathway can lead to aberrant immune activation, associated with autoimmunity, contact hypersensitivity, and allergy. The recent reports that mutations in the $\text{Nf}\kappa\text{B}$ signaling pathway are associated with excess keratinocyte death, and yet severe skin inflammation may provide an interesting model to probe this pathway further.⁹

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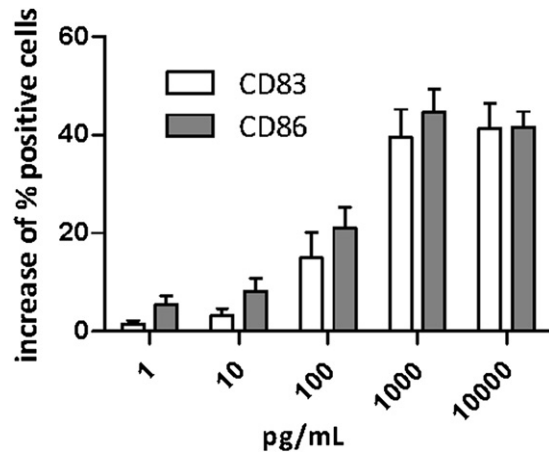


FIG E1. DCs were stimulated with increasing concentrations of IL-1 α for 20 hours. Increase in median fluorescent intensity relative to unstimulated cells (mean + SEM, $n \geq 4$).

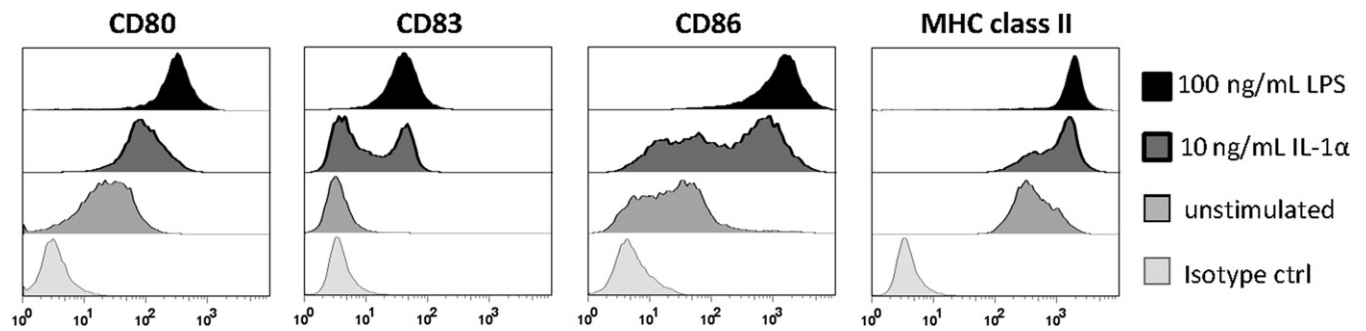


FIG E2. DCs were stimulated with IL-1 α or LPS for 20 hours. Representative fluorescence-activated cell sorting plots are shown.

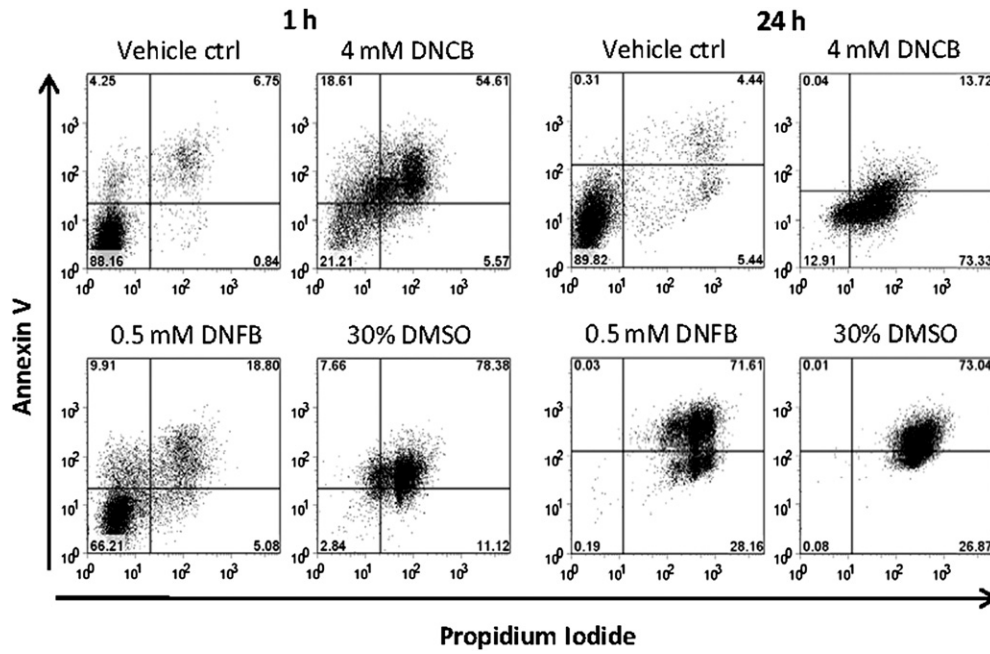


FIG E3. Chemically treated HaCaT cells were cultured for 1 or 24 hours before staining with annexin V and propidium iodide.