

Induction of IL-15 Messenger RNA and Protein in Human Blood-Derived Dendritic Cells

A Role for IL-15 in Attraction of T Cells¹

Helmut Jonuleit,* Kirsten Wiedemann,* Gabriele Müller,* Joachim Degwert,[†] Udo Hoppe,[†] Jürgen Knop,* and Alexander H. Enk^{2*}

IL-15 is a pleiotropic cytokine with IL-2-like functions. As IL-15 was shown to be mitogenic for T cells, we wondered whether human blood-derived dendritic cells (DC), as the primary stimulators of T cell responses, are able to produce IL-15. To test our hypothesis, DC were grown under serum-free conditions from human peripheral blood using granulocyte-macrophage CSF and IL-4. Cultures were assayed for IL-15 mRNA production at various times by semiquantitative reverse transcription-PCR. Low baseline signals were detected from days 0 to 5 of culture. A significant increase was detected from days 5 to 9 of the culture. When DC were further enriched by immunomagnetic beads to >98% purity as determined by CD83 staining, IL-15 mRNA signals were exclusively found in the CD83⁺ fraction. This increase in mRNA signals was paralleled by IL-15 protein release from days 9 to 12 as detected by CTLL-2 assay and ELISA. In addition, protein levels were increased >10-fold by adding paramagnetic beads to the cultures, thereby inducing phagocytic activity. Furthermore, DC supernatants were tested for chemokinetic and chemotactic activities for T cells in a checkerboard filter assay. It was shown that supernatants express chemokinetic and chemotactic activity for T cells. This activity was blocked almost completely by addition of an anti-IL-15 mAb. Our data show that human blood DC contain IL-15 mRNA and produce functional protein that is induced in culture. Protein release is triggered by phagocytic activity. Furthermore, DC-derived IL-15 has chemotactic and chemokinetic activities for T cells, suggesting a role for IL-15 as an attractant of T cells during the initial DC/T cell interaction. *The Journal of Immunology*, 1997, 158: 2610–2615.

The recently described cytokine IL-15 was originally discovered because of the capacity of culture supernatants from two cell lines, CV-1/EBNA and the HTLV-1-associated HuT-102, to stimulate proliferation of the cytokine-dependent murine T cell line CTLL-2 (1–3). Since then, IL-15 was shown to share a number of biological activities with IL-2. This includes the induction of proliferation of CD4⁺CD8[−] as well as subsets of T cells (1). IL-15 also stimulates the proliferation of NK cells and acts as a costimulator with IL-12 to facilitate the production of IFN- γ and TNF- α by these cells (4). IL-15 addition also promotes the induction of cytolytic effector cells, including cytotoxic T cells and lymphokine-activated killer cells (1). Interestingly, IL-15 acts as a potent chemoattractant for T cells, inducing chemokinesis as well as chemotaxis in these cells (5, 6). Furthermore, IL-15 induces proliferation and Ig synthesis by human tonsillar B cells stimulated by CD40 ligand or an immobilized Ab to IgM (7).

In contrast to the IL-2-like effects that IL-15 exerts on T cells, the cellular sources of the two cytokines are virtually distinct.

Whereas IL-2 is a strict T cell product, IL-15 is not produced by resting or activated normal T cells. IL-15 mRNA is found in many epithelial cells, as well as placenta, skeletal muscle, kidney, lung, heart, fibroblasts, and activated monocytes (1, 8). In skin, IL-15 mRNA is found in keratinocytes as well as Langerhans cells (9, 10). Although IL-15 mRNA transcript appears to be widely expressed (1), documentation of IL-15 protein production has been difficult, especially in resting or activated murine monocytes (8). Cultured human bone marrow stromal cells express IL-15 protein (11), as do activated human monocytes (12). These data suggest that IL-15 mRNA production and IL-15 protein secretion are differentially regulated not only at the transcriptional or message stabilization level, but also on the level of protein synthesis. In fact, certain nucleotide sequences in the 5' untranslated region of IL-15 mRNA have been shown to reduce the efficiency of mRNA translation (13).

To date, only one report has dealt with the production of IL-15 mRNA by dendritic cells (DC). Blauvelt et al. recently showed that epidermal Langerhans cells as well as cultured CD1a⁺ cells from human blood contain IL-15 mRNA (10). No IL-15 protein was detected by these investigators, and no functional information was provided about IL-15 production by DC. We, therefore, wondered whether appropriate culture and/or stimulation conditions might stimulate blood-derived CD83⁺ DC to release functional IL-15 protein. Our data demonstrate that blood-derived DC grown in GM-CSF³ and IL-4 up-regulate IL-15 mRNA in culture and start

*Clinical Research Unit, Department of Dermatology, University of Mainz, Mainz; and [†]Paul Gerson Unna Research Center, Beiersdorf, Hamburg, Germany

Received for publication July 10, 1996. Accepted for publication December 10, 1996.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¹ This work was supported by the Deutsche Forschungsgemeinschaft.

² Address correspondence and reprint requests to Dr. Alexander H. Enk, Department of Dermatology, University of Mainz, Langenbeckstr. 1, 55101 Mainz, Germany.

³ Abbreviations used in this paper: GM-CSF, granulocyte-macrophage colony-stimulating factor; SACS, *Staphylococcus aureus* Cowan strain; TAE, Tris acetate and EDTA; DIG, digoxigenin; RT-PCR, reverse transcription-polymerase chain reaction.

to release a low baseline level of IL-15 protein after 9 to 12 days. This low level of IL-15 production by CD83⁺ DC can be dramatically up-regulated when DC are induced to phagocytose immunomagnetic particles. Furthermore, the IL-15 protein released by DC under these conditions was shown to induce chemotaxis and chemokinesis in human T cells.

Materials and Methods

Cell culture

Blood-derived DC were prepared according to a modified protocol originally described by Romani et al. (14). Briefly, whole blood was heparinized and separated by Ficoll gradient. PBMC fractions were then depleted of T cells and B cells using immunomagnetic beads coated with anti-CD2 and anti-CD19 mAb (Dyna, Oslo, Norway). The remaining cells were cultured in x-vivo-15 (BioWhittaker, Gaithersburg, MD) in 6-well plates (Costar, Cambridge, MA) for 7 to 9 days. Cultures were supplemented with 1000 U/ml human IL-4 (PBH, Hannover, Germany) and 800 U/ml human GM-CSF (Leukomax, Sandoz, Nürnberg, Germany), as well as 1% autologous plasma. Cells were fed with fresh medium every 2 days. On day 7, non-adherent cells were rinsed off the plates and resuspended in fresh medium with GM-CSF and IL-4 containing 10 ng/ml TNF (R&D Systems, Wiesbaden, Germany) and 10 ng/ml IL-1 (R&D Systems).

For stimulation experiments, either 1 ng/ml PMA (Sigma Chemical Co., St. Louis, MO), a 1/5000 dilution of *Staphylococcus aureus* Cowan strain (SACS; Sigma Chemical Co.), or five uncoated immunomagnetic beads/DC (Dyna; 4.5 μ m) were added at the times indicated. Supernatants were harvested 24 h later. For detection of IL-15 protein, supernatants were fivefold concentrated with Centricon-10 concentrators as indicated by the manufacturer (Amicon, Witten, Germany).

FACS analysis

After culture, cells were subjected to analysis by FACS using the following mAb: anti-CD2 (Immunotech, Hamburg, Germany), anti-CD14 (Immunotech), anti-CD19 (Immunotech), anti-CD56 (Immunotech), anti-CD80 (Immunotech), anti-CD83 (DC-specific, kindly provided by Dr. Tedder, Duke University, Raleigh, NC), anti-CD86 (PharMingen, Palo Alto, CA), anti-HLA-DR (Serotec, Camon, Wiesbaden, Germany), and anti-HLA-DQ (Immunotech). As secondary reagents, FITC-conjugated goat anti-rat IgG (Jackson ImmunoResearch, West Grove, PA) and phycoerythrin-conjugated donkey anti-mouse IgG (Jackson ImmunoResearch) were used.

PCR analysis and liquid hybridization

Semiquantitative PCR analysis was performed as previously described (15). Briefly, total RNA was extracted from DC by RNeasy B (Wak Chemie, Bad Homburg, Germany). Afterward, RNA was quantified by spectrophotometric analysis. PCR was performed following standard protocols with conditions of 60 s at 94°C, 90 s at 55°C, and 90 s at 72°C. Primer sequences were as follows: IL-15, 5'-CCATAGCCAACTCTCTTC; and IL-15, 3'-GGTGAACATCACTTCTG. β -Actin sequences have been previously published (15). The primer concentrations and amounts of RNA were carefully titrated in pilot experiments to achieve linearity and allow for quantitative comparison of resulting signals as previously described (15). The probe sequence was: GCATTCATGTCTTCATTTGGG CTGTTTC. PCR products were separated by agarose gel electrophoresis at 60 V for 2 h in 1.5% agarose in 1 \times TAE buffer containing 0.4 μ g/ml ethidium bromide. To validate the predicted size of the PCR amplicates, a pBR322/*A**lu*I digest was run in parallel as a DNA m.w. marker. To confirm the origin of the amplified PCR products, a nonisotopic Southern blot hybridization with internal digoxigenin (DIG)-tailed oligonucleotide probes was performed. By a neutral Southern transfer technique, PCR products were blotted onto a positively charged nylon membrane (Boehringer Mannheim, Mannheim, Germany). The DNA was immobilized by baking the membrane (120°C, 30 min). The oligonucleotide probes were tailed using the DIG oligonucleotide tailing kit (Boehringer Mannheim). After Southern blot hybridization, the DIG-labeled hybridization products were detected by ELISA using an anti-DIG-alkaline phosphatase conjugate and a luminescence substrate. Visualization was achieved by exposure of the membrane to an x-ray film.

Protein analysis

The commercially available IL-15 ELISA (Laboserv, Giessen, Germany) was used as indicated by the manufacturer. For analysis in the CTLL-2 assay, DC supernatants were removed after various culture periods as indicated. CTLL cells (2×10^3 cells/50 μ l) were dispensed into 96-well

flat-bottom plates containing 50 μ l of medium titrated from supernatants. Triplicate cultures were assayed. As a control, saturating levels of anti-IL-15 mAb, anti-IL-2 mAb, or anti-IL-4 mAb (PharMingen) were added to the cultures. Titrated amounts of rIL-15 served as positive controls (not shown). Cultures were incubated for 48 h, and 1 μ Ci [³H]TdR was added during the last 12 h of culture. Thymidine incorporation was measured by liquid scintillation counting.

Checkerboard filter assay

To assay for the chemotactic and chemokinetic activities of DC supernatants, T lymphocytes were prepared from blood using standard protocols and immunomagnetic beads (Dyna). T cells were applied to filter paper and allowed to migrate for 2 h into cellulose ester filters (8- μ m pore size; Sartorius, Göttingen, Germany) with a checkerboard of titrations of DC-supernatant concentrations above and below the filter. In this assay, T cell migration is dependent on two effects caused by substances in the supernatants. One is chemokinesis, that is random movement induced by a substance. Chemokinesis does not depend on a gradient and, therefore, is reflected by the numbers printed in bold type on the diagonal of Table 1. The other is chemotaxis, meaning directed migration following a gradient. Chemotaxis is shown on the horizontal and vertical axes. After fixation and staining, the distance migrated by the leading front of the cells was measured. For specificity controls, supernatants were preabsorbed with a blocking anti-human IL-15 mAb. The distance expected of cells responding to absolute concentration alone was calculated as outlined previously (16). Distances greater than expected in positive gradients and smaller than expected in negative gradients suggest a chemotactic response.

Results

IL-15 mRNA is induced in DC during culture

Peripheral blood PBMC were prepared as described in *Materials and Methods* and grown for 11 to 13 days in culture. The phenotype of cultured cells on day 9 of culture shows all characteristics of DC, with high levels of CD83, MHC class II, CD80, and CD86 being expressed on the cell surface (Fig. 1). In a time-course experiment, IL-15 mRNA expression was analyzed by RT-PCR from days 1 to 12 of culture. Whereas very little IL-15 mRNA could be detected at early points in the culture period, a strong induction of IL-15 mRNA was observed starting from day 7 (Fig. 2). In contrast, β -actin control signals were not affected by the culture conditions. To confirm DC as the cellular source of the IL-15 mRNA signal, DC were further enriched to >98% purity by immunomagnetic beads using CD83 as a marker. RT-PCR analysis showed that the IL-15 mRNA signal almost exclusively originated in the CD83⁺ fraction (Fig. 3). Thus, DC were shown to be sources of IL-15 mRNA signals.

IL-15 protein is released in DC cultures

As the presence of IL-15 mRNA did not necessarily reflect the synthesis of functional IL-15 protein, supernatants of DC cultures were assayed for IL-15 production by CTLL-2 assay and by ELISA. Low levels of IL-15 production were detected by ELISA starting from days 6 to 7 of culture, reaching peak strength on days 8 to 9 of culture (Figs. 4 and 5). The functional relevance of the IL-15 protein released by DC was especially demonstrated by the induction of T cell proliferation in the CTLL-2 assay (Fig. 5). This proliferation could be blocked specifically by addition of blocking quantities of an anti-IL-15-specific mAb, but not by anti-IL-2- or anti-IL-4-specific mAb (Fig. 5). Thus, low levels of functional IL-15 protein were released by DC in culture.

Induction of IL-15 protein production by phagocytosis

To determine which stimuli would trigger the release of IL-15 protein into the culture, DC cultures were exposed to various stimuli on days 7 and 8. Stimuli included PMA, SACS, and induction of phagocytosis by addition of paramagnetic microbeads. Twenty-four hours later, supernatants were harvested and analyzed for

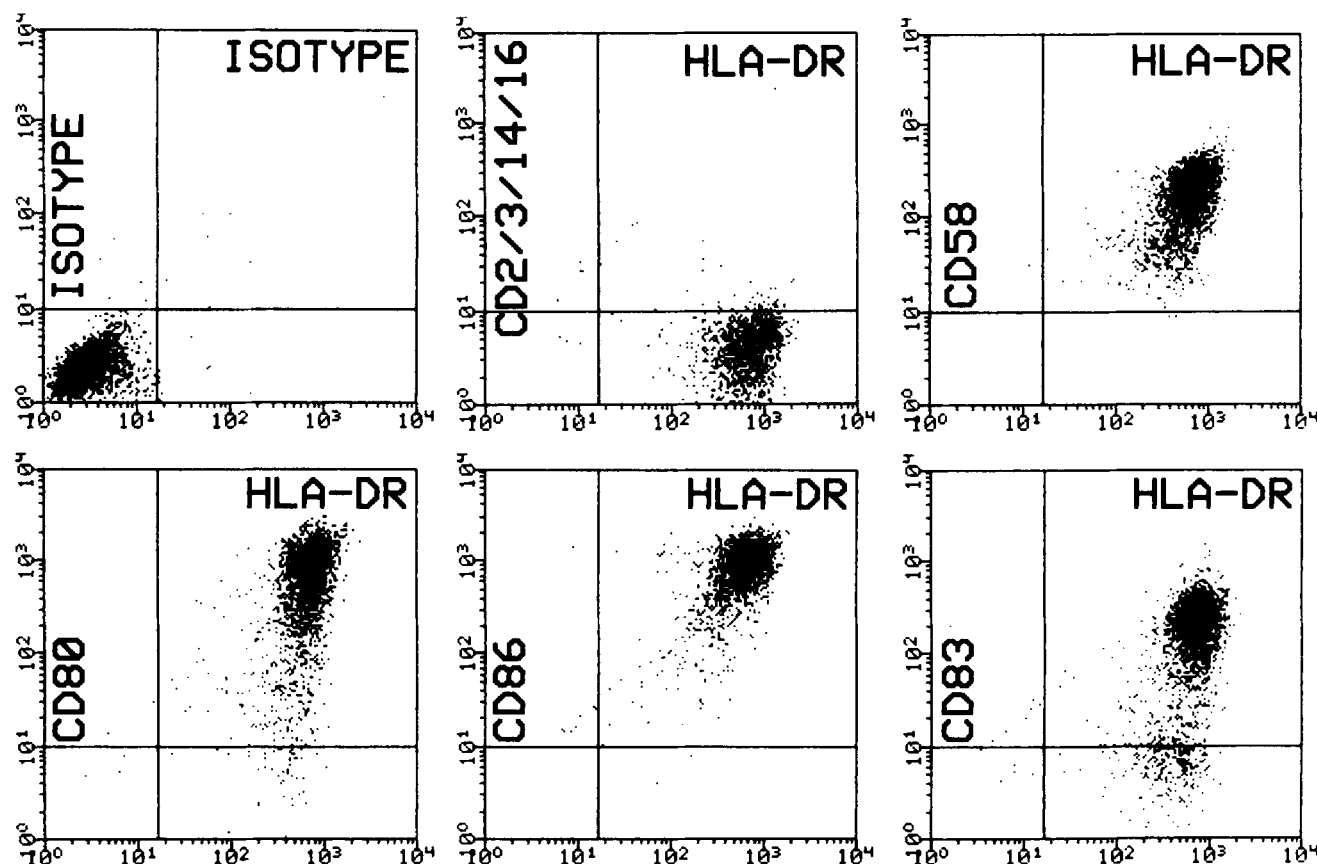


FIGURE 1. Phenotype of cultured DC after 9 days of culture. Cells were cultured as described in *Materials and Methods* and analyzed by FACSscan on day 9. Stains are as indicated in the FACS profiles, with the exception of the control mixture in which CD19 and CD56 were included as well as CD2/3/14/16.

IL-15 production by ELISA. It is shown that induction of phagocytosis by immunomagnetic beads was the most potent stimulus for IL-15 up-regulation in vitro (Figs. 4 and 5). Also, stimulation of DC by SACS was shown to enhance IL-15 protein production (Figs. 4 and 5). The data demonstrate that release of IL-15 protein can be induced by induction of phagocytosis or by stimulation with bacterial Ags. It should be added, however, that although beads were observed by microscopy within DC, it cannot be ruled out that mere binding of beads or bacterial particles is enough for induction of IL-15 protein production.

Induction of T cell chemotaxis by DC-derived IL-15

To address the question of whether DC-derived IL-15 would be able to induce chemotaxis or chemokinesis in T cells, a checkerboard analysis was performed using supernatant dilutions of stimulated DC cultures. Supernatants were obtained after stimulation of DC on either day 7 or day 8 of culture with identical results. The data shown are from day 7 cultures. Supernatant dilutions were applied below and above the filter, and cell migration was calculated after 2 h. Cell migration increased as the concentration of the supernatant (and the IL-15 concentration) was increased (along the diagonal from the *upper left* to the *lower right*, Table I). Preabsorption of the supernatants with neutralizing anti-IL-15 mAb blocked T cell migration down to baseline levels (not shown). In positive gradients (*above the diagonal*), migration was greater than that calculated on the basis of a response to absolute concentrations alone (= chemotaxis); in negative gradients (*below the diagonal*), it was lower (Table I). These results suggest that DC

supernatants induce both a chemokinetic and a chemotactic response and that the causative agent is DC-derived IL-15.

Discussion

Our results demonstrate that IL-15 mRNA is induced in blood-derived DC during culture in GM-CSF and IL-4. The origin of IL-15 mRNA is shown by enrichment of CD83⁺ genuine mature DC with immunomagnetic beads. Also, the induction of IL-15 mRNA is paralleled by a moderate induction of IL-15 protein in the culture supernatants. This moderate release of IL-15 protein can be triggered by induction of phagocytosis using paramagnetic beads or stimulation with bacterial products such as SACS. Additionally, DC-derived IL-15 is shown to exert chemokinetic and chemotactic effects on human T cells, an effect that can be abrogated by preincubation with anti-IL-15 mAb. Therefore, it is concluded that DC can release functional IL-15 following stimulation.

DC are the premier APC of the human body. Their capacity to capture Ag and to induce primary T cell responses is more advanced and far more developed than those of any other APC (17). This capacity to induce T cell responses partly originates from the fact that DC express a multiplicity of surface markers on their surface that facilitate contact and stimulation of T cells. Among them are an abundance of MHC class I and II molecules, adhesion molecules such as ICAM or LFA, or costimulatory signals such as CD80, CD86, or heat stable Ag (17). Besides these membrane-bound factors, DC also are able to release soluble mediators such as IL-1 and IL-12 that further enhance the capacity of these APC

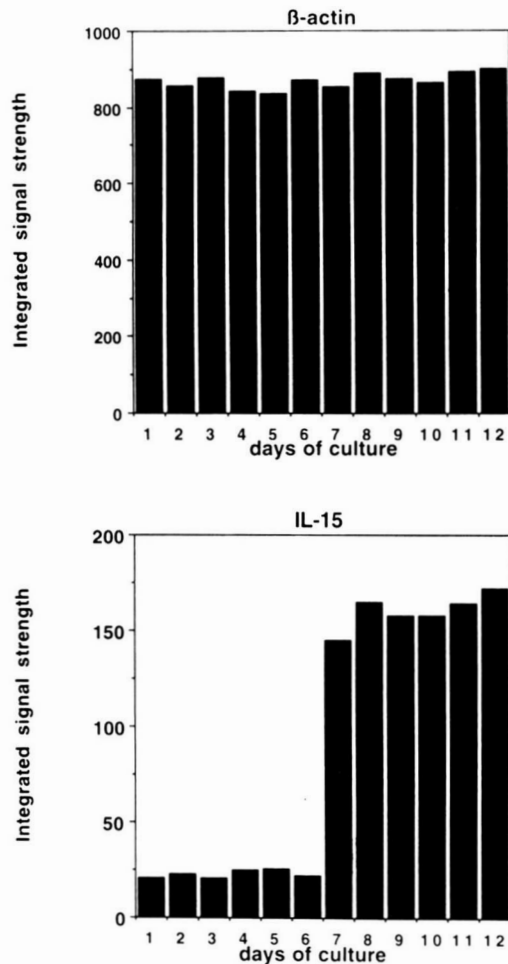


FIGURE 2. Analysis of IL-15 mRNA expression during culture of blood DC. Cells were cultured as described in *Materials and Methods*. At the times indicated, mRNA was extracted and analyzed by semi-quantitative RT-PCR and Southern blotting. Afterward signals were analyzed by optometric scanning. The y-axes show integrated signal strength for IL-15 and β -actin mRNA signals.

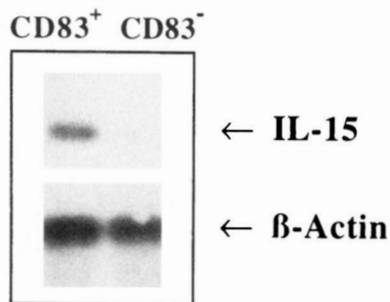


FIGURE 3. CD83⁺ DC express abundant IL-15 mRNA signals. DC were cultured as described and enriched for CD83⁺ DC by immunomagnetic beads to a purity of >98%. Afterward, mRNA was extracted from both fractions (depleted fraction containing <0.5% CD83⁺ DC) and analyzed for IL-15 and β -actin mRNA by RT-PCR and Southern blotting.

to induce proliferation and differentiation of T cells (18, 19). Both, IL-1 as well as IL-12 have been shown to be able to induce proliferation in T cells, and IL-12 is supposed to be the prime mediator for the development of Th1 cells (20).

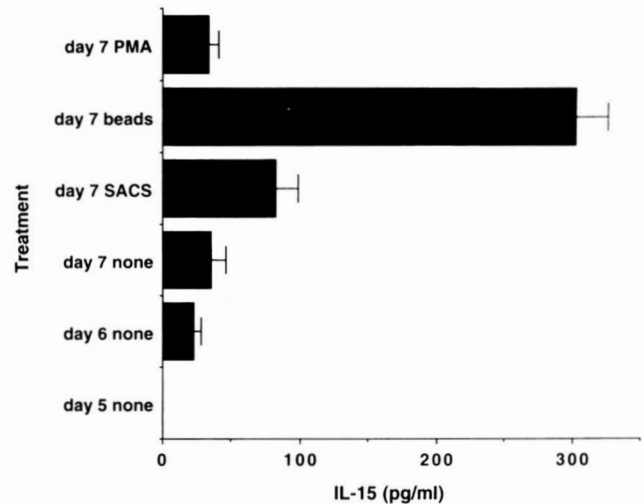


FIGURE 4. Release of IL-15 protein into the culture medium. Supernatants from DC cultures were removed after the points indicated. Before analysis, DC were stimulated with SACS, immunomagnetic beads, or PMA or were left unstimulated. ELISAs were performed following the manufacturer's instruction. The data represent IL-15 protein levels in fivefold concentrated supernatants that were normalized to a 1 \times concentration.

As DC are so important in inducing T cell responses, it is not surprising that they also produce factors that are directly T cell mitogenic, such as IL-15. IL-15 is a cytokine with IL-2-like functions that uses parts of the IL-2R as well as an IL-15-specific receptor to exert its function (2, 21–23). As IL-2 is not produced by DC, IL-15 could be a substitute molecule that DC use instead. Especially our experiments showing that IL-15 production is induced in DC by phagocytosis point to a role for IL-15 in mediating the initial T cell/DC contact. As phagocytosis, e.g., of bacteria, is a first step in the induction of a T cell response against bacterial Ags, the simultaneous release of a signal that attracts a partner T cell would be ideal for the initiation of an immune response. Interestingly, resting human monocytes (another major APC) and PBMC do not produce IL-15 protein. After activation with bacterial products such as LPS or human herpesvirus-6, however, both cell populations up-regulate IL-15 mRNA and produce IL-15 protein. The secretion of IL-15 *in vivo* following bacterial or viral infections may, therefore, represent a first line host defense mechanism (12, 24).

With regard to DC, it might, therefore, be that DC following activation by Ags and induction of Ag processing and presentation also start to release IL-15 protein. This IL-15 might then serve as a potent chemoattractant for T lymphocytes that will start to migrate toward the activated DC and thereby facilitate the initial contact between DC and T cells. Although this is certainly not the only mechanism that DC use to achieve contact with the appropriate T cell, it certainly is helpful for the speed of induction of any immune response.

The finding that IL-15 is primarily induced by phagocytosis or binding of particles or after stimulation with bacterial Ags is also in good agreement with our time-course analysis of IL-15 protein induction. As demonstrated, IL-15 mRNA and protein are only induced at later stages of DC culture, that is in more mature DC. This maturation stage in culture usually reflects the natural development of DC that have been stimulated *in vivo* with Ags in the periphery. Day 7 DC in culture are still considered to be rather immature DC. Following stimulation (e.g., with bacteria), these

FIGURE 5. Production of functional IL-15 protein by DC. DC culture supernatants were harvested at the points indicated, either with or without prior stimulation of cultures and fivefold concentrated (results are again normalized to a 1× concentration). CTLL assays were performed as described. As a specificity control, neutralizing amounts of anti-IL-2, anti-IL-4, and anti-IL-15 mAb were added to CTLL assays before analysis. Thymidine incorporation was determined after 48 h.

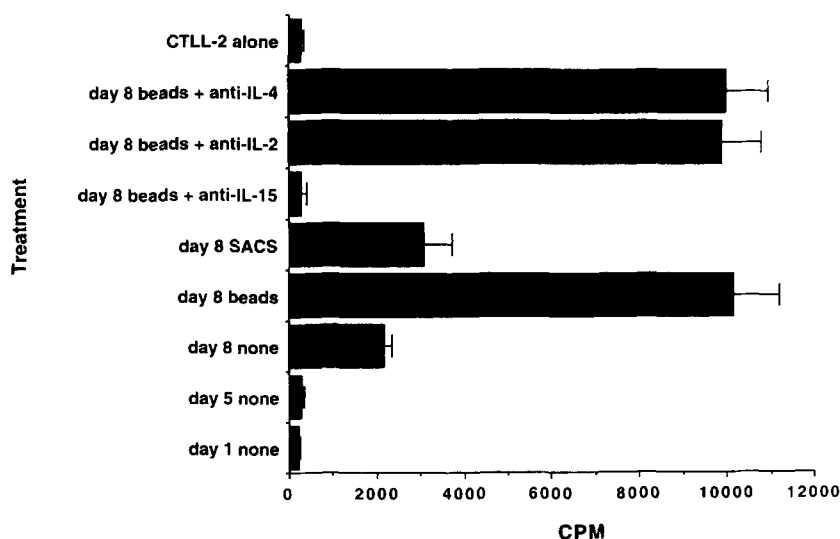


Table 1. Distance migrated (μm in 2 h) by leading front of cells (mean \pm SEM)

Supernatant Dilutions Above Filter ^a	Supernatant Dilutions Below Filter ^b				
	\emptyset^c	1:100	1:10	1:1	^d 1:1 + Anti-IL-15
\emptyset	19 \pm 3	48 \pm 2 (21) ^e	60 \pm 4 (28)	65 \pm 4 (34)	21 \pm 4
1:100	31 \pm 4 (32)	33 \pm 3	47 \pm 5 (31)	57 \pm 4 (36)	34 \pm 5
1:10	39 \pm 3 (51)	34 \pm 4 (44)	45 \pm 4	55 \pm 5 (46)	43 \pm 4
1:1	46 \pm 4 (52)	45 \pm 5 (53)	48 \pm 4 (52)	52 \pm 4	47 \pm 4

^a Supernatant dilutions above filter represent the media in which T cells were resuspended and applied to the filter.

^b Supernatants below filter indicate concentrations underneath the filter paper. Bold numbers = chemokinesis.

^c \emptyset represents control culture medium alone.

^d 1:1 + Anti-IL-15 represents addition of saturating amounts of blocking anti-IL-15 mAb (20 $\mu\text{g}/\text{ml}$) to undiluted samples of the supernatants. Further details of the experimental setting are explained in the *Materials and Methods* section.

^e Figures in parentheses show the calculated distance that would be migrated if cells responded to the absolute concentration of IL-15, but not to the gradient (14).

DC will then start to migrate to regional lymph nodes, express the appropriate Ag, and then stimulate T cells. IL-15 might, therefore, play an important role in the induction of these T cell responses (17).

Our data are also in accord with a prior report by Blauvelt et al. (10). These investigators showed that blood-derived DC as well as Langerhans cells from suction blisters contain IL-15 mRNA. No IL-15 protein was detected by these investigators. For their blood-DC studies these investigators used a serum-containing protocol generating DC with GM-CSF and IL-4. Cells were analyzed on day 7, and no time course experiments were performed. In agreement with our results, IL-15 mRNA was detected in the CD14⁺ fraction of these cultures. No IL-15 protein could be detected. There are several possible explanations for this. First, even in our own cultures very low amounts of IL-15 protein are detectable on day 7, so that the sensitivity of the assay used for detection is crucial. Second, Blauvelt et al. did not stimulate their DC as was performed in our experiments, and third, the different culture protocols used to generate blood-DC might result in cells with variable capacities to produce IL-15 protein in culture.

In aggregate, our experiments show that DC-derived IL-15 is capable of inducing chemotaxis and chemokinesis in T cells. Further in vivo studies will now have to demonstrate whether DC use this factor as an adjuvant to facilitate the primary contact with T lymphocytes.

Acknowledgment

The authors thank Mrs. Pirch for help with the figures.

References

- Grabstein, K. H., J. Eisenman, K. Shanebeck, S. Rauch, S. Srinivasan, V. Fung, C. Beers, J. Richardson, M. A. Schoenborn, and M. Ahdieh. 1994. Cloning of a T cell growth factor that interacts with the β -chain of the interleukin-2 receptor. *Science* 264:965.
- Bamford, R. N., A. J. Grant, J. D. Burton, C. Peters, G. Kurys, C. K. Goldman, J. Brennan, E. Roessler, and T. A. Waldmann. 1994. The interleukin (IL) 2 receptor beta chain is shared by IL-2 and a cytokine, provisionally designated IL-T, that stimulates T-cell proliferation and the induction of lymphokine-activated killer cells. *Proc. Natl. Acad. Sci. USA* 91:4940.
- Burton, J. D., R. N. Bamford, C. Peters, A. J. Grant, G. Kurys, C. K. Goldman, J. Brennan, E. Roessler, and T. A. Waldmann. 1994. A lymphokine, provisionally designated interleukin T and produced by a human adult T-cell leukemia line, stimulates T-cell proliferation and the induction of lymphokine-activated killer cells. *Proc. Natl. Acad. Sci. USA* 91:4935.
- Carson, W. E., J. G. Giri, M. J. Lindemann, M. L. Linett, M. Ahdieh, R. Paxton, D. Anderson, J. Eisenman, K. Grabstein, and M. A. Caligiuri. 1994. Interleukin (IL) 15 is a novel cytokine that activates human natural killer cells via components of the IL-2 receptor. *J. Exp. Med.* 180:1395.
- Wilkinson, P. C., and F. Y. Liew. 1995. Chemoattraction of human blood T lymphocytes by interleukin-15. *J. Exp. Med.* 181:1255.
- McInnes, I. B., J. al Mughaies, M. Field, B. P. Leung, F. P. Huang, R. Dixon, R. D. Sturrock, P. C. Wilkinson, and F. Y. Liew. 1996. The role of interleukin-15 in T-cell migration and activation in rheumatoid arthritis. *Nat. Med.* 2:175.
- Armitage, R. J., B. M. Macduff, J. Eisenman, R. Paxton, and K. H. Grabstein. 1995. IL-15 has stimulatory activity for the induction of B cell proliferation and differentiation. *J. Immunol.* 154:483.
- Doherty, T. M., R. A. Seder, and A. Sher. 1996. Induction and regulation of IL-15 expression in murine macrophages. *J. Immunol.* 156:735.
- Mohamadizadeh, M., A. Takashima, I. Dougherty, J. Knop, P. R. Bergstresser, and P. D. J. Cruz. 1995. Ultraviolet B radiation up-regulates the expression of IL-15 in human skin. *J. Immunol.* 155:4492.
- Blauvelt, A., H. Asada, V. Klaus-Kovtun, D. Altman, D. R. Lucey, and S. I. Katz. 1996. Interleukin-15 mRNA is expressed by human keratinocytes, Langerhans cells, and blood-derived dendritic cells and is downregulated by UV-B radiation. *J. Invest. Dermatol.* 106:1047.

11. Mrozek, E., P. Anderson, and M. A. Caligiuri. 1996. Role of interleukin-15 in the development of human CD56⁺ natural killer cells from CD34⁺ hematopoietic progenitor cells. *Blood* 87:2632.
12. Carson, W. E., M. E. Ross, R. A. Baiocchi, M. J. Marien, N. Boiani, K. Grabstein, and M. A. Caligiuri. 1995. Endogenous production of interleukin 15 by activated human monocytes is critical for optimal production of interferon- γ by natural killer cells in vitro. *J. Clin. Invest.* 96:2578.
13. Tagaya, Y., R. N. Bamford, A. P. DeFilippis, and T. A. Waldmann. 1996. IL-15: a pleiotropic cytokine with diverse receptor/signaling pathways whose expression is controlled at multiple levels. *Immunity* 4:329.
14. Romani, N., S. Gruner, D. Brang, E. Kämpgen, A. Lenz, B. Trockenbacher, G. Konwalinka, P. O. Fritsch, R. M. Steinman, and G. Schuler. 1994. Proliferating dendritic cell progenitors in human blood. *J. Exp. Med.* 18:83.
15. Enk, A. H., and S. I. Katz. 1992. Early molecular events in the induction phase of contact sensitivity. *Proc. Natl. Acad. Sci. USA* 89:1398.
16. Zigmond, S. H., and J. G. Hirsch. 1973. Leukocyte locomotion and chemotaxis. *J. Exp. Med.* 137:387.
17. Steinman, R. M. 1991. The dendritic cell system and its role in immunogenicity. *Annu. Rev. Immunol.* 9:271.
18. Enk, A. H., V. L. Angeloni, M. C. Udey, and S. I. Katz. 1993. An essential role for Langerhans-cell-derived IL-1 β in the initiation of primary immune responses of the skin. *J. Immunol.* 150:3698.
19. Macatonia, S. E., N. A. Hosken, M. Litton, P. Vieira, C. S. Hsieh, J. A. Culpepper, M. Wysocka, G. Trinchieri, K. Murphy, and A. O'Garra. 1995. Dendritic cells produce IL-12 and direct the development of Th1 cells from naive CD4⁺ T cells. *J. Immunol.* 154:5071.
20. Heufler, C., F. Koch, U. Stanzl, G. Topar, M. Wysocka, G. Trinchieri, A. Enk, R. M. Steinman, N. Romani, and G. Schuler. 1996. IL-12 is produced by dendritic cells and mediates T helper 1 development as well as interferon-gamma production by T helper 1 cells. *Eur. J. Immunol.* 26:659.
21. Giri, J. G., M. Ahdieh, J. Eisenman, K. Shanebeck, K. Grabstein, S. Kumaki, A. Namen, L. S. Park, D. Cosman, and D. Anderson. 1994. Utilization of the beta and gamma chains of the IL-2 receptor by the novel cytokine IL-15. *EMBO J.* 13:2822.
22. Giri, J. G., S. Kumaki, M. Ahdieh, D. J. Friend, A. Loomis, K. Shanebeck, R. DuBose, D. Cosman, L. S. Park, and D. M. Anderson. 1995. Identification and cloning of a novel IL-15 binding protein that is structurally related to the alpha chain of the IL-2 receptor. *EMBO J.* 14:3654.
23. Kimura, Y., T. Takeshita, M. Kondo, N. Ishii, M. Nakamura, J. Van Snick, and K. Sugamura. 1995. Sharing of the IL-2 receptor gamma chain with the functional IL-9 receptor complex. *Int. Immunol.* 7:115.
24. Flamand, L., I. Stefanescu, and J. Menezes. 1996. Human herpesvirus-6 enhances natural killer cell cytotoxicity via IL-15. *J. Clin. Invest.* 97:1373.