Efficient Presentation of Soluble Antigen by Cultured Human Dendritic Cells Is Maintained by Granulocyte/Macrophage Colony-stimulating Factor Plus Interleukin 4 and Downregulated by Tumor Necrosis Factor α

By Federica Sallusto*† and Antonio Lanzavecchia*

From the *Basel Institute for Immunology, CH-4005, Basel, Switzerland; and the †Department of Immunology, Istituto Superiore di Sanità, 00161, Rome, Italy

Summary

Using granulocyte/macrophage colony-stimulating factor (GM-CSF) and interleukin 4 we have established dendritic cell (DC) lines from blood mononuclear cells that maintain the antigen capturing and processing capacity characteristic of immature dendritic cells in vivo. These cells have typical dendritic morphology, express high levels of major histocompatibility complex (MHC) class I and class II molecules, CD1, FcyRII, CD40, B7, CD44, and ICAM-1, and lack CD14. Cultured DCs are highly stimulatory in mixed leukocyte reaction (MLR) and are also capable of triggering cord blood naïve T cells. Most strikingly, these DCs are as efficient as antigen-specific B cells in presenting tetanus toxoid (TT) to specific T cell clones. Their efficiency of antigen presentation can be further enhanced by specific antibodies via FcR-mediated antigen uptake. Incubation of these cultured DCs with tumor necrosis factor α (TNF-α) or soluble CD40 ligand (CD40L) for 24 h results in an increased surface expression of MHC class I and class II molecules, B7, and ICAM-1 and in the appearance of the CD44 exon 9 splice variant (CD44-v9); by contrast, FcγRII is markedly and sometimes completely downregulated. The functional consequences of the short contact with TNF-α are an increased T cell stimulatory capacity in MLR, but a 10-fold decrease in presentation of soluble TT and a 100-fold decrease in presentation of TT-immunoglobulin G complexes.

Dendritic cells (DCs) play a critical role in antigen presentation in vivo (1). They exist in two stages of maturation. As immature cells, DCs are scattered throughout the body in nonlymphoid organs, where they appear to exert a sentinel function. They pick up and process antigen and subsequently move to the T-dependent areas of secondary lymphoid organs. During this process of maturation, they lose antigen-capturing capacity and become mature immunostimulatory DCs that trigger naïve T cells recirculating through these areas (2, 3).

A similar maturation process occurs spontaneously when Langerhans cells (LCs, which represent immature DCs in skin) are cultured in vitro (4, 5). Under these conditions LCs rapidly lose the capacity to pick up and process soluble antigen, but acquire high T cell costimulatory capacity. Thus, both in vivo and in vitro studies suggest that antigen capture/processing and immunostimulation are the property of DCs at different stages of maturation.

The mechanism of antigen uptake determines the efficiency of presentation of soluble antigens on class II molecules (6). The pinocytic activity of DCs has been reported to be at least as high as that of other APCs (7). In addition, splenic DCs and LCs express FcγRII, which is lost when these cells mature in vitro (4, 8). Although DCs are able to present soluble antigen (5, 9–11), their efficiency has not been compared to that of other APCs. A general consensus has emerged that DCs may actually be rather inefficient in presenting soluble antigens and it has been argued that, for this reason, efficient presentation in vivo may be an exclusive property of antigen-specific B cells (12).

A common progenitor for granulocytes, macrophages, and DCs has been identified in mouse bone marrow (13) as a MHC class II negative cell that can develop into the three different myeloid pathways under the aegis of GM-CSF. Proliferating precursors of DCs that can be expanded into DC lines in vitro with GM-CSF are present both in mouse bone marrow (14) and peripheral blood (15). To date, human DC lines have...
been generated only from CD34+ precursors isolated from cord blood (16) or bone marrow (17) using a combination of GM-CSF and TNF-α.

There are several reasons for wishing to establish in vitro cultures of immature DCs. First, to exploit their antigen-presenting capacity; second to compare them with other APCs for this function; third, to identify the signals that modulate antigen capturing and presenting function. Here we describe a method to culture DCs from human peripheral blood, such that the phenotypic and functional characteristics of immature DCs are retained. These cells are indeed as efficient as antigen-specific B cells and can use FcγRII to further increase uptake of antigen in antigen–antibody complexes. Maturation of these cells can be induced by TNF-α, resulting in upregulation of their capacity to stimulate naive allogeneic T cells and downregulation of their capacity to present soluble antigen.

Materials and Methods

Media and Reagents. The medium used throughout was RPMI 1640 supplemented with 2 mM l-glutamine, 1% nonessential amino acids, 1% pyruvate, 50 µg/ml kanamycin, 5 × 10−5 M 2-ME (Gibco Laboratories, Grand Island, NY) and 10% FCS (Hyclone Laboratories, Inc., Logan, UT). Tetanus toxoid (TT) was purchased from Connaught Laboratories, Ltd. (Willowdale, Ontario, Canada). Human anti-TT antibodies (all IgG) were purified by chromatography on protein A-Sepharose from concentrated culture supernatants of EBV-B cell clones (18). Human recombinant IL-2, IL-4, and GM-CSF were produced in our laboratory by PCR cloning and expression in the myeloma expression system described by Traunecker et al. (19). The concentration of IL-4 and GM-CSF were determined using commercial ELISA assays. Purified human recombinant GM-CSF and TNF-α were a generous gift of Dr. R. W. Brockhaus (Hoffmann-La Roche, Basel, Switzerland). A soluble chimeric fusion protein between the mouse CD8 α-chain and the antigen.

Antigen Presentation Assays. TT-specific EBV-B cell clones and TT-specific T cell clones were isolated and maintained as previously described (18). T cell clone AS1.15 recognizes a TT determinant corresponding to residues 947–967 in association with DP4. To measure the efficiency of presentation of a soluble antigen, 2 × 10⁴ TT-specific T cells were cultured with 5 × 10⁴ cultured DCs (3,000 rad), 2 × 10⁴ irradiated autologous EBV-B cell clones (6,000 rad) or 10⁵ autologous PBMCs (3,000 rad) in the presence of different concentrations of TT in 200 µl RPMI-10% FCS in flat-bottom microplates. The cultures were set up in the presence or absence of a fixed concentration of a mixture of six different anti-IT IgG antibodies (0.5 µg/ml each). [3H]Thymidine incorporation was measured after 48 h. TT and anti-TT antibodies were allowed to react for 1 h before the addition of APCs and T cells. In some experiments, total PBMCs (1.5 × 10⁵) or polyclonal short-term TT-specific T cell lines (2 × 10⁴) were used as a source of TT-specific cells. [3H]Thymidine incorporation was measured on days 5 and 2, respectively.

Results

Culture Conditions for the Generation of DCs with Antigen-presenting Capacity. Adherent cells or the light density Percoll fraction from PBMCs were depleted of T and B cells and cultured in RPMI–FCS supplemented with various combinations of GM-CSF, IL-4, and TNF-α. The cell yields, surface phenotype and functional properties of cells grown with different cytokine combinations are shown in Table 1. It is evident that a combination of GM-CSF and IL-4 provided the best conditions for the generation of cells with the characteristic phenotype and functional properties of DCs (high expression of CD11c, class II and B7, and high stimulatory capacity in allogeneic and autologous MLR). Furthermore cells from GM-CSF + IL-4–dependent cultures were the most efficient at presenting soluble antigen TT to specific T cell
clones. Cells grown with a combination of GM-CSF and TNF-α (16) were inferior to those obtained with GM-CSF + IL-4, especially for presentation of soluble antigen. We therefore used DCs from GM-CSF + IL-4–dependent culture in subsequent experiments.

In a typical experiment, after ~7 d of culture with GM-CSF + IL-4, 50–80% of the cells appear as loosely adherent clumps or isolated floating cells with the typical dendritic morphology (see an example in Fig. 1) and motility, as assessed by time lapse videorecording (data not shown). Analysis of surface markers (Table 1 and Fig. 2) showed that the large cells were homogeneous and expressed high levels of MHC class I and class II molecules, CD1a, CD1b and CD1c, FcγRII, ICAM-1, CD11b, CD11c, CD40, B7, and CD33. CD14 was either low or negative in different preparations. Furthermore, DCs were positive for li, LFA-1, LFA-3, and CD44 and negative for FcγRI and FcγRIII (see also Table 2). Anti-CD3 and anti-CD19 antibodies were always used as control and found negative. Cell growth rapidly slowed down after the first 3–4 wk, but viable cells could be maintained in culture with occasional feeding for up to 3 mo.

Stimulatory Capacity of DCs. Cultured DCs were compared with PBMCs for their capacity to stimulate alloreactive T cells. Different numbers of DCs or PBMCs from the same donor were cultured with a fixed number of allogeneic T cells. Fig. 3 a shows that as few as 50 DCs could trigger a substantial response; on a per cell basis, DCs were 300-fold more effective than PBMCs in stimulating adult T cells. It is interesting to note that only DCs but not PBMCs could trigger cord blood T cells (Fig. 3 b). The observation that cord blood T cells, which are entirely naive, could be stimulated only by DCs underlines the specialized role of DCs for T cell priming.

Efficient Presentation of Soluble Antigen and Antigen–Antibody Complexes by Immature DCs. To evaluate the capacity of DCs to present a soluble antigen, we compared DCs, PBMCs, and antigen-specific B cells for their capacity to present TT to a TT-specific T cell clone. To evaluate the possible effect of FcγR in enhancing capture of antigen–antibody complexes, the cultures were set up in the presence or absence of a fixed concentration of anti-TT IgG antibodies.

As evident from Fig. 4, the efficiency of presentation, as measured from the TT concentration necessary to give 50% of maximum response, varies with the type of APC. DCs (Fig. 4 a) were the most effective APCs, since they could present TT at a concentration of 10^-10 M, while PBMCs (Fig. 4 b) and nonspecific B cells (Fig. 4 c) required antigen concentrations higher than 10^-8 M. Furthermore, in the presence of anti-TT antibodies, the efficiency of DCs increased at least 100-fold and a significant proliferative response was

Figure 1. Typical appearance of DC cultures on day 15.
Table 1.  **Cell Yield, Surface Phenotype, Stimulatory, and Antigen-presenting Capacity of Light Density Mononuclear Cells Cultured with Various Cytokine Combinations**

|               | Alone | GM-CSF | IL-4 | TNF-α | GM-CSF | TNF-α | GM-CSF | IL-4 |
|---------------|-------|--------|------|-------|--------|-------|--------|------|
| **Cell yield (%)** |       |        |      |       |        |       |        |      |
| Day 4         | 23    | 68     | 23   | 43    | 57     | 84    |        |      |
| Day 8         | 6     | 114    | 25   | 46    | 74     | 99    |        |      |
| Day 20        | 2     | 152    | 14   | 64    | 90     | 125   |        |      |
| **Surface markers** |     |        |      |       |        |       |        |      |
| CD1a          | -     | -      | -    | -     | ±      | +     |        |      |
| CD1b          | -     | ±      | -    | -     | ±      | +     |        |      |
| CD1c          | -     | -      | -    | -     | ±      | +     |        |      |
| DR            | ++    | ++     | ++   | ++    | ++     | ++    |        |      |
| DQ            | -     | -      | +    | -     | -      | +     |        |      |
| Class I       | ++    | ++     | ++   | ++    | ++     | ++    |        |      |
| CD40          | -     | +      | -    | +     | +      | +     |        |      |
| CD40c         | +     | ++     | ++   | +     | ++     | ++    |        |      |
| ICAM-1        | +     | +      | +    | +     | +      | +     |        |      |
| CD14          | +     | ±      | ±    | +     | -      | -     |        |      |
| **Stimulation and antigen presentation** |     |        |      |       |        |       |        |      |
| Allogeneic MLR |       |        |      |       |        |       |        |      |
| Maximum response | nd  | 32 ± 2 | 40.9 ± 2.7 | 33.4 ± 1.7 | 53.1 ± 3 | 68.1 ± 2.2 |
| No. cells for 50% response | 6,000 | 4,000 | 12,000 | 3,000 | 600 |
| Autologous MLR |       |        |      |       |        |       |        |      |
| Maximum response | nd  | <1    | <1   | <1   | <1    | 10.1 ± 2 |
| TT presentation |       |        |      |       |        |       |        |      |
| Maximum response | nd  | 89.4 ± 4.5 | nd | nd | 8.2 ± 2.1 | 134.6 ± 4 |
| ng/ml TT 50% response | 100 |      | >10⁴ |      | 10 |

* Cell yield at days 4, 8, and 20 expressed as percent of input cells.
† The various cell populations were tested on day 8 for their capacity to stimulate in allogeneic and autologous MLR. The maximum response and the number of cells required for stimulating 50% of the maximum response are shown.
§ 5 × 10⁶ cells from the various cell populations were tested on day 10 for their capacity to present different concentrations of TT to a TT-specific T cell clone. The maximum response and the concentration of TT required for stimulating 50% of the maximum response are shown.
‡ cpm × 10⁻³ ± SD.

obtained at TT concentrations of 10⁻¹⁴ M. When compared with PBMCs, DCs were at least 100-fold more efficient, both in the absence and in the presence of anti-TT antibody (Fig. 4 b). Finally, DCs were as efficient as some antigen-specific B cells (Fig. 4 c) and, in the presence of soluble antibodies, appeared to be the most efficient APC for soluble antigens.

We also compared DCs and PBMCs for their capacity to present TT to autologous peripheral blood T cells and to polyclonal short-term TT-specific lines. As evident from Fig. 5, a and b, DCs were again more powerful than PBMCs, both in terms of maximum response and amount of TT required. The shape of the dose-response curve may be due to the presence of T cells with different sensitivities to antigen and thus may reflect the ability of DCs to stimulate a higher number of specific T cells.

**Modulation of Surface Phenotype and Antigen-presenting Function by CD40L and TNF-α.** The above results indicated that DCs obtained from GM-CSF + IL-4 cultures shared many prop-
properties with immature DCs such as LCs, at least with respect to surface phenotype and presentation of soluble antigen. We therefore asked whether these cells might be induced to mature in culture and whether this maturation would affect antigen-presenting capacity.

We tested the effect of TNF-α and CD40L, which represent two effector molecules of natural and acquired immunity. As shown in Table 2, DCs underwent a rapid change in surface phenotype upon incubation with TNF-α or CD40L. By 24 h, surface MHC class I and class II molecules increased two- to threefold, while Ii expression was reduced by approximately half. ICAM-1 expression also increased, a fact that was presumably responsible for the spontaneous cell aggregation observed. B7 and CD40 were also upregulated, whereas FcγRII was rapidly and sometimes completely downregulated. Interestingly, treatment with TNF-α increased CD44 expression and induced the appearance of a new splice variant carrying exon 9 (22).

Some of these changes resemble those occurring in vivo when LCs move from skin to lymph nodes (4). We therefore asked what consequence these changes might have on the capacity of DCs to stimulate T cells and to present soluble antigens.

Figure 3. Cultured DCs are highly stimulatory in MLR and are the only cells capable of triggering cord blood T cells. Adult peripheral blood (a) or cord blood mononuclear (b) cells were cultured with different numbers of allogeneic PBMCs (●, ■) or DCs (○, □) from the same donor. The proliferative response was measured on day 5.
Figure 4. Cultured DCs are the most efficient APCs for presentation of soluble antigen as such or complexed with IgG antibodies. The three panels represent the proliferative response of the same T cell clone to different concentrations of TT in the presence or absence of a fixed concentration of anti-TT IgG antibodies and different irradiated APCs. (a): 5 x 10^6 DCs in the absence (□) or presence (■) of anti-TT antibody. (b): 10^6 PBMCs in the absence (○) or presence (■) of anti-TT antibody. (c): 2 x 10^4 EBV-B cells from two TT-specific clones (○, △) in the absence of anti-TT antibody or a nonspecific polyclonal line in the absence (△) or presence (▲) of anti-TT antibody. Proliferative response was measured on day 2.

Discussion

The availability of immature DCs is instrumental for studying the mechanisms of antigen capture and processing by these cells, as well as to identify signals that modulate this function. In this study we have shown that it is possible to grow in vitro human cell lines with many of the characteristics of immature DCs. The two most striking findings are the highly efficient presentation of soluble antigen by these cell lines, and their rapid response to TNF-α leading to up-regulation of adhesion and costimulatory molecules and down-regulation of antigen-capturing and -processing capacity.

Our DC lines differ from those described by Caux et al. (16) in two important aspects: the use of adult PBMCs (23) rather than cord blood precursors and the use of IL-4 rather than TNF-α. Indeed adult adherent cells grown with GM-CSF + TNF-α have lower stimulatory capacity and are unable to present soluble antigen (Table 1), a fact that can be explained by the capacity of TNF-α to regulate antigen-presenting function (Fig. 6). Our DC lines were generated from adult peripheral blood and require IL-4 in addition to
Table 2. Phenotypic Changes of DCs Cultured for 24 h with TNF-α or CD40L

|                      | GM-CSF+IL4 + TNF-α* + CD40L† |
|----------------------|-----------------------------|
| Second Ab            | 28s                         |
| CD1a                 | 369                         |
| CD1b                 | 200                         |
| CD1c                 | 295                         |
| DR                   | 1,555                       |
| DQ                   | 549                         |
| DP                   | 129                         |
| Ll                   | 245                         |
| MHC class I          | 991                         |
| Fc'RII               | 1,684                       |
| B7                   | 84                          |
| CD40                 | 673                         |
| CD11c                | 534                         |
| ICAM-1               | 228                         |
| LFA-1                | 550                         |
| LFA-3                | 219                         |
| CD44                 | 845                         |
| CD44-v9              | 62                          |
| CD14                 | 75                          |

* DCs were incubated for 24 h with 10 ng/ml TNF-α.
† DCs were incubated for 24 h with CD40L-mouse CD8 at a saturating concentration as determined by staining with anti-mouse CD8.

GM-CSF to maintain the immature, antigen presentation competent state. These conditions have been previously shown to increase CD1 expression on adherent cells (21, 24). It is not clear what role IL-4 may play, but it is interesting to speculate it may antagonize the effect of TNF-α and other maturation-inducing signals. Whether IL-4 or other cytokines may play a physiological role in maintaining the immature DC pool in vivo is a matter of speculation.

The identification of GM-CSF/IL-4-expanded cells as DCs was based on three well-established and accepted criteria (25, 26): first, their typical morphology and motility; second, their surface phenotype, with high expression of CD1, MHC class I and class II, II, FcRRII, B7, CD40, ICAM-1, LFA-3, and CD11c; and third, their high stimulatory capacity for naive T cells (27-29). In this regard, it is worth noting that only DCs but not PBMCs could activate cord blood T cells. This finding reinforces the notion that DCs are the only cells capable of triggering naive T cells and is an apparent contrast with a report that adult CD45RA+ naive T cells can be stimulated by allogeneic PBMCs (30). It is possible, however, that either cord blood cells have higher requirements for costimulation than adult T cells, or that the alloreactive response by adult CD45RA+ T cells may involve memory cells that have reverted to the CD45RA+ phenotype, but retain the capacity to respond to nonprofessional or semiprofessional APCs (31, 32).

Presentation of soluble antigen by DCs has been reported to be rather inefficient in the sense that relatively high concentrations of antigen were required (10⁻⁶ and 10⁻⁷ M), comparable to those required by other nonantigen-specific APCs (4, 9-11). In contrast, we found that DCs cultured with GM-CSF + IL-4 can present TT at concentrations of 10⁻¹⁰ M and are therefore 100-300-fold more efficient than nonspecific B cells or PBMCs. These DCs are actually comparable to antigen-specific B cells, which can use membrane Ig for antigen capture. Furthermore, in the presence of immune complexes, DCs become even more efficient than antigen-specific B cells, being able to present TT at the extraordinary low concentration of 10⁻¹² M. This is the most efficient presentation of soluble antigen reported to date.

This highly efficient presentation of soluble antigen depends on the preservation of the immature phenotype, since
it is lost when DCs are induced to mature by TNF-α. It is thus possible that the antigen-presenting capacity of DCs has been previously underestimated because the immature FcγR⁺ cells may have been lost or induced to mature during the isolation procedure.

Several mechanisms may contribute to the efficient antigen presentation of DCs: first, their capacity for clustering T cells in an antigen-independent fashion (33); second, the expression of high levels of MHC molecules, allowing presentation of more T cell determinants; third, the high expression of adhesion and costimulatory molecules and the low surface charge (34, 35), which may lower the number of determinants required for T cell activation (36, 37); and fourth, the high level of fluid-phase pinocytosis (7) and the expression of functional FcγR (11, 38).

A striking finding is the response of DCs to TNF-α and CD40L. Within 24 h, surface expression of MHC class II, ICAM-1, LFA-3, CD40, and B7 increases two- to threefold, whereas expression of Ii and FcγRII decreases. The functional consequences are an increased T cell stimulatory capacity in MLR, but a 10-fold decrease in presentation of soluble TT and a 100-fold decrease in presentation of TT-IgG complexes. The effect on class II molecules and Ii is of particular interest. Preliminary experiments indicate that the increase in surface class II expression is not accompanied by an increase in class II biosynthesis, suggesting an effect of TNF-α at the posttranslational level. Indeed, staining for intracellular class II molecules and Ii is of particular interest. Thus, there are differences in the mechanism that lead to downregulation of antigen presenting capacity in cultured DCs and in fresh LCs. Whereas in both cases FcγRII is downregulated, a downregulation of class II synthesis is observed only in LCs (39, 40). Further work is required to identify the level of this regulation and the signals involved (41, 42).

It is interesting to discuss our results in the context of the well known maturation pathway of LCs. It has been shown that LCs form a reservoir of immature DCs that, upon antigenic stimulation, resume their migratory behavior and move to the draining lymph nodes, where they arrive as mature DCs (1, 43). A similar maturation process is known to occur spontaneously when these cells are cultured in vitro (4). On the basis of our results it is interesting to hypothesize that TNF-α may play a physiological role in vivo in the induction of migration and maturation (44, 45). Local production of TNF-α at sites of encounter with "infectious" antigen (46) may induce maturation of DCs and their migration from tissues into secondary lymphoid organs. It is interesting to note that TNF-α induces the appearance of a CD44 isoform carrying the v9 exon, which may be involved in controlling migratory behavior (47). The role of CD40L may be limited to a later stage, when DC localize in T-dependent areas of lymph nodes.

Whereas local production of TNF-α may play a physiological role in regulating antigen presentation by DCs, high systemic levels of TNF-α, such as in malignancies or chronic inflammatory diseases, may be detrimental (48). Too much TNF-α may cause generalized immunosuppression by inducing all DCs to mature and lose the capacity to present new incoming antigens.

We thank Manfred Brockhaus for providing GM-CSF and TNF-α, Peter Lane for providing soluble CD40L, Charles Mackay for providing anti-CD44 variant antibodies, and Kristian Hannestad, Alexandra Livingstone, and Charles Mackay for critical reading and comments.

The Basel Institute for Immunology was founded and is supported by F. Hoffmann-La Roche & Co. Ltd., Basel, Switzerland.

Address correspondence to Dr. Antonio Lanzavecchia, Basel Institute for Immunology, Grenzacherstrasse 487, CH-4005 Basel, Switzerland.

Received for publication 7 September 1993 and in revised form 17 November 1993.

References

1. Steinman, R.M. 1991. The dendritic cell system and its role in immunogenicity. Annu. Rev. Immunol. 9:271.
2. Romani, N., and G. Schuler. 1992. The immunologic properties of epidermal Langerhans cells as part of the dendritic cell system. Springer. Semin. Immunopathol. 13:265.
3. Austyn, J.M. 1992. Antigen uptake and presentation by dendritic leukocytes. Semin. Immunol. 4:227.
4. Schuler, G., and R.M. Steinman. 1985. Murine epidermal Langerhans cells mature into potent immunostimulatory dendritic cells in vitro. J. Exp. Med. 161:526.
5. Romani, N., S. Koide, M. Crowley, M. Witmer-Pack, A.M. Livingstone, C.G. Fathman, K. Inaba, and R.M. Steinman. 1989. Presentation of exogenous protein antigens by dendritic cells to T cell clones. Intact protein is presented best by immature epidermal Langerhans cells. J. Exp. Med. 169:1169.
6. Lanzavecchia, A. 1990. Receptor-mediated antigen uptake and its effect on antigen presentation to class II restricted T lymphocytes. Annu. Rev. Immunol. 8:773.
7. Levine, T.P., and B.M. Chain. 1992. Endocytosis by antigen presenting cells: dendritic cells are as endocytically active as...
other antigen presenting cells. Proc. Natl. Acad. Sci. USA. 89:8342.
8. Girolomoni, G., J.C. Simon, P.R. Bergstreser, and P.J. Cruz. 1990. Freshly isolated spleen dendritic cells and epidermal Langerhans cells undergo similar phenotypic and functional changes during short-term culture. J. Immunol. 145:2820.
9. De Brujin, M.L.H., J.D. Nieland, C.V. Harding, and C.J.M. Melief. 1992. Processing and presentation of intact hen egg-white lysozyme by dendritic cells. Eur. J. Immunol. 22:2347.
10. Aiba, S., and S.I. Kats. 1991. The ability of cultured Langerhans cells to process and present protein antigen is MHC dependent. J. Immunol. 146:2479.
11. Harkiss, G.D., J. Hopkins, and I. McConnell. 1990. Uptake of antigen by afferent lymph dendritic cells mediated by antibodies. Eur. J. Immunol. 20:2367.
12. Mamula, M.J., and C.A. Janeway. 1993. Do B cells drive the diversification of immune responses? Immunity Today. 14:151.
13. Inaba, K., M. Deguchi, K. Hagi, R. Yasumizu, S. Ikehara, S. Muramatsu, and R.M. Steinman. 1993. Granulocytes, macrophages, and dendritic cells arise from a common major histocompatibility complex class II-negative progenitor in mouse bone marrow. Proc. Natl. Acad. Sci. USA. 90:3038.
14. Inaba, K., M. Inaba, N. Romani, H. Aya, M. Deguchi, S. Ikehara, S. Muramatsu, and R.M. Steinman. 1992. Generation of large numbers of dendritic cells from mouse bone marrow cultures supplemented with granulocyte/macrophage colony-stimulating factor. J. Exp. Med. 167:1693.
15. Inaba, K., R.M. Steinman, M. Wittmer Pack, H. Aya, M. Inaba, T. Sudo, S. Wolpe, and G. Schuler. 1992. Identification of proliferating dendritic cell precursors in mouse blood. J. Exp. Med. 175:1157.
16. Caux, C., D.C. Dezutter, D. Schmitt, and J. Banchereau. 1992. GM-CSF and TNF-alpha cooperate in the generation of dendritic Langerhans cells. Nature (Lond.). 360:258.
17. Reid, C.D., A. Stackpoole, A. Meager, and J. Tkerpae. 1992. Interactions of tumour necrosis factor with granulocyte-macrophage colony-stimulating factor and other cytokines in the regulation of dendritic cell growth in vitro from early bipotent CD34+ progenitors in human bone marrow. J. Immunol. 149:2681.
18. Lanzavecchia, A. 1985. Antigen-specific interaction between T and B cells. Nature (Lond.). 314:537.
19. Trauwehner, A., F. Oliveri, and K. Kajjalainen. 1991. Myeloma-based expression system for production of large mammalian proteins. Trends Biotechnol. 9:109.
20. Lane, P., T. Broker, S. Hubele, E. Padovan, A. Lanzavecchia, and F. MccInnon. 1993. Soluble CD40 ligand can replace the normal T cell-derived CD40 ligand signal to B cells in T cell-dependent activation. J. Exp. Med. 177:1209.
21. Porcelli, S., C.T. Morita, and M.B. Brenner. 1992. CD1b restricts the response of human CD4-8- T lymphocytes to a microbial antigen. Nature (Lond.). 360:593.
22. Güntert, U. 1994. CD44: a multitude of isoforms with diverse functions. Curr. Top. Microbiol. Immunol. In press.
23. Rossi, G., N. Heveker, B. Thiele, H. Gelderblom, and F. Steinbach. 1992. Development of a Langerhans cell phenotype from peripheral blood monocytes. Immunol. Lett. 31:189.
24. Kasserer, W., T. Baumrucker, O. Majdic, W. Knapp, and H. Stockinger. 1993. CD1 molecule expression on human monocytes induced by granulocyte-macrophage colony-stimulating factor. J. Immunol. 150:579.
25. Freundental, P.S., and R.M. Steinman. 1990. The distinct surface of human blood dendritic cells, as observed after an improved isolation method. Proc. Natl. Acad. Sci. USA. 87:7698.
26. Thomas, R., L.S. Davis, and P.E. Lipsky. 1993. Isolation and characterization of human peripheral blood dendritic cells. J. Immunol. 150:821.
27. Croft, M., D.D. Duncan, and S.L. Swain. 1992. Response of naive antigen-specific CD4+ T cells in vitro: characteristics and antigen-presenting cell requirements. J. Exp. Med. 176:1431.
28. Inaba, K., G. Shuler, M.D. Witmer, J. Valinsky, B. Atassi, and R.M. Steinman. 1986. Immunologic properties of purified epidermal Langerhans cells. Distinct requirements for stimulation of unprimed and sensitized T lymphocytes. J. Exp. Med. 164:605.
29. Seder, R.A., W.E. Paul, M.M. Davis, and B. Fazekas de St. Groth. 1992. The presence of interleukin 4 during in vitro priming determines the lymphokine-producing potential of CD4+ T cells from T cell receptor transgenic mice. J. Exp. Med. 176:1091.
30. Merkenschlager, M., L. Terry, R. Edwards, and P.C.L. Beverley. 1988. Limiting dilution analysis of proliferative responses in human lymphocyte populations defined by the monoclonal antibody UCHL1: implications for differential CD45 expression in T cell memory formation. Eur. J. Immunol. 18:1653.
31. Bell, E.B., and S.M. Sparshott. 1990. Interconversion of CD45R subsets of CD4 T cell in vivo. Nature (Lond.). 348:163.
32. Michie, C.A., A. McLean, C. Alcock, and P.C.L. Beverley. 1992. Lifespan of human lymphocyte subset defined by CD45 isoforms. Nature (Lond.). 360:264.
33. Inaba, K., N. Romani, and R.M. Steinman. 1989. An antigen-independent contact mechanism as an early step in T cell-proliferative responses to dendritic cells. J. Exp. Med. 170:527.
34. Sprent, J., and M. Schaefer. 1990. Antigen-presenting cells for CD4+ T cells. Immunity. Rev. 117:213.
35. Neefjes, J.J., M.L.H. De Brujin, C.J.P. Boog, J.D. Nieland, J. Boes, C.J.M. Melief, and H.L. Ploegh. 1990. N-linked glycan modification on antigen-presenting cells restores an allospecific cytotoxic T cell response. J. Exp. Med. 171:583.
36. Demotz, S., H.M. Grey, and A. Sette. 1990. The minimal number of class II MHC-antigen complexes needed for T cell activation. Science (Wash. DC). 249:1028.
37. Harding, C.V., and E.R. Unanue. 1990. Quantitation of antigen-presenting cell MHC class II/peptide complexes necessary for T-cell stimulation. Nature (Lond.). 346:574.
38. Zaghouani, H., R. Steinman, R. Nonacs, H. Shah, W. Gerhard, and C. Bona. 1993. Presentation of a viral T cell epitope expressed in the CD8+ region of a self immunoglobulin molecules. Science (Wash. DC). 259:224.
39. Kampgen, E., N. Koch, F. Koch, P. Stoger, C. Heufler, G. Schuler, and N. Romani. 1991. Class II major histocompatibility complex molecules of murine dendritic cells: synthesis, sialylation of invariant chain, and antigen processing capacity are down-regulated upon culture. Proc. Natl. Acad. Sci. USA. 88:3014.
40. Puré, E., K. Inaba, M.T. Crowley, L. Tardelli, P.M. Witmer-Pack, G. Ruberti, G. Fathman, and R.M. Steinman. 1990. Antigen processing by epidermal Langerhans cells correlates with the level of biosynthesis of major histocompatibility complex class II molecules and expression of invariant chain. J. Exp. Med. 172:1459.
41. Holt, P.G., J. Oliver, N. Bilyk, C. McMenamin, P.G. McMenamin, G. Kraal, and T. Thepen. 1993. Downregulation of the antigen presenting cell function(s) of pulmonary dendritic cells in vivo by resident alveolar macrophages. J. Exp. Med. 177:397.
42. Koch, F., C. Heufler, E. Kämpgen, D. Schnerweiss, G. Böck, and G. Schuler. 1990. Tumor necrosis factor α maintains the viability of murine epidermal Langerhans cells in culture, but in contrast to granulocyte/macrophage colony-stimulating factor, without inducing their functional maturation. *J. Exp. Med.* 171:159.

43. Austyn, J.M., and C.P. Laesen. 1990. Migration patterns of dendritic leukocytes. Implications for transplantation. *Transplantation (Baltimore)*. 49:1.

44. Cumberbatch, M., and I. Kimber. 1992. Dermal tumour necrosis factor-alpha induces dendritic cell migration to draining lymph nodes, and possibly provides one stimulus for Langerhans' cell migration. *Immunology*. 75:257.

45. Simon, J.C., D. Edelbaum, P.D. Cruz, Jr., A. Kapp, J. Krutmann, E. Schopf, and P.R. Bergstresser. 1992. Tumor necrosis factor-alpha (TNF-alpha) distorts the antigen-presenting function of epidermal Langerhans cells. *Arch. Dermatol. Res.* 284(69): (Abstr.)

46. Janeway, C.J. 1992. The immune system evolved to discriminate infectious nonself from noninfectious self. *Immunol. Today*. 13:11.

47. Günthert, U., M. Hofmann, W. Rudy, S. Reber, M. Zöller, I. Haussmann, S. Matzku, A. Wendel, H. Ponta, and P. Herrlich. 1991. A new variant of glycoprotein CD44 confers metastatic potential to rat carcinoma cells. *Cell*. 65:13.

48. Tazi, A., F. Bouchonnet, M. Grandsaigne, L. Boumsell, A.J. Hance, and P. Soler. 1993. Evidence that granulocyte macrophage-colony-stimulating factor regulates the distribution and differentiated state of dendritic cells/Langerhans cells in human lung and lung cancers. *J. Clin. Invest.* 91:566.