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Cytokines in the generation and maturation of dendritic cells: recent advances

Abstract

Dendritic cells (DCs) are extremely efficient antigen presenting cells (APCs) that are potent stimulators of both T and B cell-mediated immune responses. Although DCs are normally present in very small numbers in the peripheral blood (PB), recent advances have made it possible to generate relatively large numbers of cells in culture. DCs can be differentiated in vitro from various cellular sources, including bone marrow (BM), cord blood (CB) and PB mononuclear cells (PBMCs). Although a wide variety of conditions have been

reported to be able to support DC generation, the majority of research and clinical protocols to date differentiate DCs from precursors using granulocyte-macrophage colony stimulating factor (GM-CSF) in combination with either tumor necrosis factor-(TNF-)alpha or interleukin (IL)-4. However, a diverse array of cytokines has been shown to be able to induce DC differentiation under a variety of conditions. According to recent reports, cytokines such as IL-2, IL-6, IL-7, IL-13, IL-15 and hepatocyte growth factor (HGF), in combination or even, in some cases, alone, can contribute to the generation of DCs from either monocytes or CD34+ cells. Although the majority of cytokine combinations include GM-CSF, some do not. For example, Flt3 ligand (FL), in conjuction with IL-6 (in the absence of GM-CSF), has been reported to be able to induce DC differentiation from BM cells in a murine system. Other agents can play a dual role in DC activity. CD40 ligand (CD40L), as a single agent, has been shown to be able to generate DCs from PB monocytes, while numerous other reports have also demonstrated its role as a potent maturation factor. In contrast, for other cytokines such as IL-16 or IL-17, although there is no data for a role in DC generation, they have been reported to be involved in promoting DC maturation in vitro as defined by upregulation of costimulatory molecules, major histocompatibility complex (MHC) antigens and antigen presenting/T lymphocyte stimulatory capacity. Furthermore, cytokines such as stem cell factor (SCF) and FL have been shown to dramatically enhance in vivo DC recovery. The wide variety of cytokines and conditions that have been shown to be able to influence DC differentiation and activity to amply demonstrate the extreme heterogeneity found in the DC population, something that is reflected in the diverse phenotypes, functions and ontogeny displayed by DCs. This diversity may account for the large number of roles that have been attributed to DCs in the development and function of the immune system and, in turn, emphasizes the potential as well as the challenges of modifying specific aspects of the immune response system by manipulating specific DC subpopulations.

Full text

INTRODUCTION

Dendritic cells are professional APCs that play a crucial role in the initiation and regulation of both humoral and cellular immune responses. These cells were first described many years ago by Langerhans in 1868 but they were not referred

to as "dendritic cells" until 1973 by Steinman and Cohn, who were able to identify, isolate and characterize DCs from mice spleen [1]. Their role in the regulation of immune responses was subsequently established a number of years later, in a series of studies in the late 1970s and early 1980s [2, 3]. Unfortunately, research in the area was severely hampered by the fact that, under normal physiological conditions, only very low numbers of dendritic cells were present in the peripheral blood. Furthermore, specific phenotypic markers, to allow definitive identification and isolation of pure DC populations were lacking.

However, DC research underwent a significant renaissance with the development of procedures to obtain relatively large numbers of cells with DC-like characteristics *via* extended culture of PB, BM or CB precursor cells in the presence of specific cytokines, typically GM-CSF in conjunction with either TNF-alpha or IL-4 [4-6], resulting in expansion and/or differentiation of several cell types, including those in the DC lineage. The *in vitro* generation of DCs from two specific precursor populations has been particularly well characterized: PB monocytes and $CD34^+$ stem cells (derived from BM, mobilized PB or CB). Both macrophages and DCs can be differentiated from blood monocytes, the ultimate outcome being dependent on the culture conditions. While cells cultured with GM-CSF consistently differentiate to a macrophage phenotype that exhibits adherence to plastic and cell surface expression of CD14, monocytes cultured with GM-CSF and IL-4 rapidly lose CD14 expression, become nonadherent and acquire morphology typical of DCs [7]. Alternatively, DCs can also be generated from BM and granulocyte colony stimulating factor (G-CSF) or GM-CSF mobilized PB $CD34^+$ hematopoietic progenitor cells (HPCs) by culture in the presence of GM-CSF and TNF-alpha. Interestingly, even HPC-derived DCs show some heterogeneity (PB $CD34^+$ cells may give rise to greater numbers of fully functional DCs compared to their BM counterparts) suggesting heterogeneity within the stem cell population [8].

This renaissance rekindled interest in DC research that resulted in significant progress on several fronts, including the development of specific antibodies [9, 10] and protocols that enabled identification and isolation of dendritic cells. The development of culture methods and identification of specific markers therefore, represent two major milestones that have, in turn, fostered a huge body of research investigating both basic DC biology and function as well as translational clinical applications of DCs for immunotherapy of disease.

With the tremendous advances in the understanding of DC biology, one fact that has become abundantly clear is that the so-called "dendritic cells" actually represent a widely heterogeneous population of cells. A number of criteria have been used to delineate or define these DC populations. Initial work focused on classifying DCs largely on the basis of their phenotypic profile and these criteria are still widely used to either identify specific DC populations or determine their maturation or activation state. Immature monocyte-derived dendritic cells (MoDCs) express intermediate levels of the cell surface markers CD40, CD80, CD86, and HLA-DR on their surface. In addition, immature DCs possess a number of mechanisms by which antigen uptake and internalization is achieved, including macropinocytosis [11], Fc-mediated uptake and antigen-specific receptors (such as CD32, CD64, DEC-205 and macrophage mannose receptor [12]. Upon maturation by such stimulii as TNF-alpha, lipopolysaccharide (LPS) or CD40L, cell surface expression of the costimulatory CD80 and CD86 molecules as well as HLA-DR is upregulated. As well, maturation induces *de novo* expression of CD83 which is therefore used a surrogate marker for mature DCs [13]. These changes reflect the decreased antigen uptake, but enhanced presentation and T cell stimulation functions of mature DCs. Phenotypically, distinct DC subpopulations have also been defined by the expression of specific cell surface antigens in addition to CD80, CD86 and CD83. For example, CD1a molecule is expressed on DCs during certain maturational phases coincident with antigen capture and processing [14]. MoDCs have been classified as $CD1a^{+}$ and $CD1a^{-}$ DC subsets that differ in their cytokine production profiles and capacity to direct CD4 T helper (Th) cell differentiation [15]. In humans, other MoDC markers include, the beta2-integrins CD11a, CD11b, CD11c and CD18 [16]. In the mice, the DCs have been characterized as either $CD11b^{high}CD11c^{high}$ or $CD11b^{low}CD11c^{high}$ [17].

More recently, DCs have been classified on the basis of their functional characteristics, specifically as DC1, DC2 [18-21], and the more recently described DC3 [22] dendritic cells, based on their ability to induce Th1, Th2 and tolerogenic immune responses, respectively. In this regard, DCs can be classified into different subtypes based on both their cytokine production and the type of Th response that they elicit. Generally, mature MoDCs are termed DC1, produce IL-12 and polarize Th1 development. On the other hand, DCs generated from $CD34^{+}$ are generally DC2 cells, produce interferon-(IFN-)alpha and beta, and polarize Th2 cell development. Currently, no data have been

reported with regard to a DC subset that can polarize Th3 cell development (T helper cells that produce and secrete TGF-beta [23, 24]).

Finally, DCs have also been distinguished based on either their tissues of residence (such as Langerhans cells (LC) that reside in the epidermis, follicular DCs from the germinal centers in the thymus, or DCs that reside in the dermis, spleen, lymphatic system, intestine, etc.), or their ontogeny based on their derivation from a particular precursor cell type, or more generally, along specific lineages such as myeloid or lymphoid pathways.

Unfortunately, a fundamental problem with the fact that DCs have been described using a variety of criteria is that it is not immediatedly clear how data derived from a population DCs described in one manner relate to those DCs described using different parameters. Although work defining the interrelation between phenotypically, functionally and ontogenically defined DCs is underway, these relationships have not yet been fully defined and characterized. As a result, those seeking an understanding of the research in the DC field face an added a level of complexity. This has been even further confounded by an extensive amount data derived from animal studies, particularly in murine models. Since equivalent populations have not been identified for all DC subgroups, in all animal models, it is impossible to completely relate studies in one animal model to either those in other animals or to the human condition.

Studies of DC generation and maturation have been carried out using a wide range of *in vitro* and *in vivo* systems, combining research of both *in vivo* DC ontogeny and *ex vivo* differentiation and utilizing a broad array of precursor cell types in various animal models. The heterogeneity of DCs is well demonstrated by the wide variety of cytokines that have been reported in the scientific literature to play a role in either DC generation and/or maturation. Work to identify the cytokines that are able to generate DCs has yielded confusing and often contradictory reports. In this paper, we will review the recent advances surrounding the role of cytokines in dendritic cell generation and maturation. In spite of the difficulties encountered by DC researchers in relating and coordinating the huge amount of data, the basic and translational research in DC biology continues to expand, due in no small part to the potential of DCs for clinical immunotherapeutic applications.

CYTOKINE-INDUCED DIFFERENTIATION OF DCs

Granulocyte-macrophage colony-stimulating factor, interleukin-4 and tumour necrosis factor-alpha

The combination of GM-CSF with IL-4 or TNF-alpha represents, by far, the most extensively characterized and utilitized cytokine combinations that have been identified to support DC differentiation *in vitro*. GM-CSF (as well as SCF and FL), together with TNF-alpha, have been well described as being active in the differentiation of $CD34^+$ to cells with DC phenotype [5, 25, 26], whereas GM-CSF and IL-4 seem to be sufficient for culture of monocytes to DCs [27, 28].

GM-CSF has been shown to function by inducing DC progenitor expansion as well as promoting differentiation and survival of DCs [29]. In addition to inducing differentiation, TNF-alpha has been shown to strongly potentiate the proliferation of $CD34^+$ HPCs induced by either IL-3 or GM-CSF [5, 30] and to be required for the clonogenic growth of pure human DC colonies in the presence of GM-CSF [31]. Conversely, IL-4 functions to inhibit macrophage colony formation in addition to its role in inducing DC growth and maturaton from monocytes [4].

Treatment with GM-CSF and IL-4 or TNF-alpha produce DCs with an immature phenotype that display inefficient antigen presentation. Upon treatment with a number of maturative factors, DCs acquire the characteristics of mature DC, including morphology, loss of monocyte markers, loss of antigen uptake, upregulation of accessory molecules, translocation of class II MHC to the cell surface and finally a capacity to efficiently prime naive T cells [32].

Interleukin-2

Reports have indicated a role for IL-2 in inducing both human $CD34^+$ cell [33] and monocyte [34] differentiation toward a DC phenotype. Bykovskaja *et al.* [33] succeeded in inducing growth and differentiation of DCs from CB $CD34^+$ cells in cultures initiated in media supplemented with IL-2 alone or in combination with SCF and TNF-alpha, and subsequently grown with either IL-2 alone or IL-2 and SCF for 4-5 weeks. The morphology and phenotype of these DCs were similar to those cultured in the presence of GM-CSF. Similarly, Civallero *et al.* [34] recently investigated and demonstrated the differentiation of DCs from monocyte

precursors *in vitro* with IL-2, IL-4, GM-CSF and G-CSF alone or in combination. The results indicated that the differentiation was directly related to the presence of IL-2 which, in addition to playing a direct role, was able to exert a syngergistic effect with GM-CSF. However, based on their results, the authors hypothesized that IL-2 could act as a maturation factor rather than as a growth factor in the DC differentiation.

In spite of initial studies that suggested that IL-2 was not able to directly enhance DC function [35], subsequent reports have suggested a role for IL-2 in regulating DC function. Certainly, expression of the IL-2 receptors (IL-2Rs) on DCs has been demonstrated [35, 36] and furthermore, IL-2-induction of motility and accumulation of DCs in lung and skin has been shown [36]. From these results, the authors concluded that IL-2 is a potent enhancer of DC motility that may act in conjunction with other Th1 proinflammatory cytokines to attract DCs to sites of inflammation. In addition, a role in the regulation of DCs during antigen presentation has been proposed, due to observations of a synergistic effect of IL-2R activation with CD40 or MHC Class II stimulation [37].

Interleukin-3

Caux *et al.* [38] analyzed whether IL-3, in combination with TNF-alpha, could support the generation of $CD1a^+$ DCs *in vitro*. Cultures of human CB $CD34^+$ HPCs with IL-3 and TNF-alpha, yielded 11%-17% $CD1a^+$ cells while IL-3 alone did not result in significant cell numbers. Although the absolute percentage of $CD1a^+$ cells was much lower than that observed in cells cultured with GM-CSF and TNF-alpha (42%-78%), the strong proliferation induced by the combination of IL-3 and TNF-alpha resulted in an equivalent number of $CD1a^+$ cells, suggesting that IL-3 may allow expansion of DCs. More recently, Blom *et al.* [39] confirmed that IL-3 contributes (in this case, with CD40L) to the differentiation of $CD34^+$ progenitors into pre-DC2 cells.

Interleukin-3 was also found to be able to influence the function of differentiated DCs. In mice, three distinct BM-derived DC (BMDC) populations were expanded from BALB/c BM cells based on culture with GM-CSF and IL-3, IL-3 plus Th1-biasing cytokines (IL-12 and IFN-gamma) or IL-3 plus Th2-biasing cytokines (IL-4), to give rise to BMDC0, BMDC1 and BMDC2 cells respectively. While all of these cells expressed the DC-specific marker CD11c, they demonstrated differential functionality. BMDC1 cells exhibited superior T cell-stimulating

activity in allogeneic MLRs, inducing a higher frequency of IFN-gamma-producing $CD8^+$ T cells compared to BMDC2s. Moreover, BMDC1, but not BMDC2, were strong inducers of H-2(d)-specific cytotoxic T lymphocytes (CTLs). BMDC0 exhibited intermediate stimulatory activity and, when cultured with IFN-gamma, could be induced to differentiate into BMDC1-like stimulator cells, concomitant with the up-regulation of both MHC antigens and co-stimulatory molecules. In contrast, BMDC2 were refractory to further differentiation into cells with superior stimulating capacity by treatment with IFN-gamma, although this treatment enhanced MHC expression. These findings indicate that IL-3 in conjuction with Th1- and Th2-biasing cytokines (in addition to their effect on Th cell differentiation), may play a critical role in the functional skewing of DC [40].

In vivo, work by Storozynsky *et al.* [41] demonstrated that exposure to IL-3 and GM-CSF resulted in the generation of large numbers of DCs with increased T cell stimulatory capacity that were important for cross-priming and subsequent generation of tumor-reactive CTLs. These cells produced IFN-alpha and IFN-beta, in addition expressing the IL-3 receptor. To date, there is no information about the *in vivo* effect of IL-3 on human DCs.

Interleukin-6

Interestingly, studies on IL-6 have demonstrated variable effects on DC differentiation, based on precursor cell type. IL-6 was found to inhibit monocyte differentiation to DCs [42], resulting in an increased $CD14^+CD1a^-$ population concomitant with a reduced $CD14^-CD1a^+$ DC population in GM-CSF/IL-4 supplemented cultures while IL-11, leukemia inhibitory factor, oncostatin M or macrophage-colony stimulating factor (M-CSF) did not exert such an effect. This inhibitory effect was abrogated by TNF-alpha, LPS, IL-1beta, CD40L or TGF-beta1, of which TNF-alpha was the most potent, suggesting that IL-6 inhibited DC differentiation from monocytes by promoting differentiation toward a macrophage lineage that could be, in turn, modulated by other factors (*i.e.* TNF-alpha, LPS, etc.). These observations were confirmed by Chromarat *et al.* [43] who observed an IL-6-dependent switch in GM-CSF, IL-4 cultured monocyte from DCs to macrophages.

In contrast, IL-6 has been reported to induce $CD34^+$ cell expansion and differentiation into DCs in cooperation with GM-CSF [44], GM-CSF and SCF [45] or FL [46] as judged by phenotype analysis and T lymphocyte stimulatory

capacity. However, there have also been reports demonstrating an IL-6-mediated inhibition of $CD34^+$ stem cell DC differentiation. Menetier-Caux *et al.* reported that the release of IL-6 and M-CSF from renal cell carcinoma cells was able to inhibit GM-CSF/TNF-alpha-induced differentiation of $CD34^+$ cells [47], as well as monocytes [48] to DCs. These disparate effects of IL-6 on $CD34^+$ cells should not perhaps be surprising, however, in view of the fact that Menetier-Caux *et al.* reported the release of a number of soluble factors, in addition to IL-6, including M-CSF, which exert their own direct and modifying effects.

Interleukin-7

IL-7 has been shown to be able to induce DC differentiation from both $CD34^+$ thymocytes [49] and monocytes (in co-operation with GM-CSF [50]). In the latter report, investigators showed that DCs exhibiting a novel phenotype, designated G7 DCs, were generated from adherent PBMCs following 7 day culture in GM-CSF and IL-7. These cells expressed cell surface antigens typically expressed on DCs generated with GM-CSF and IL-4, including MHC class I and II, CD1a, CD11c, CD23, CD40, CD54, CD58, CD80, CD86 and CD95. However, these cells also expressed CD21 which has been reported to function as a receptor for a number of ligands, including type 2 complement, CD23, EBV and IFN-alpha. Researchers found that these DCs were at least 1-2 logs more effective in autologous and allogeneic MLR assays respectively when compared to PBMCs, and the proliferative response of CD4 and CD8 T cells (to tetanus toxoid and an EBV peptide respectively), as well as anti-EBV T cell cytotoxicity was enhanced in response to G7 DCs compared to GM-CSF/IL-4 DCs. Similarly, Li *et al.* [51] evaluated the phenotypic and functional properties of MoDCs following culture with GM-CSF alone (GM-CSF-DCs), or in combination with IL-4 (IL4-DCs) or IL-7 (IL7-DCs). Consistent with the earlier report, IL-7-DCs (as well as GM-CSF-DCs) expressed "a CD21-like molecule". The authors of these studies hypothesized that IL-7, together with GM-CSF, activates a unique signalling pathway in monocytes/macrophage resulting in differentiation to either a distinct DC subtype [50] or differentiation stage [51] that are similar but distinct from GM-CSF/IL-4-DCs.

Furthermore, work by Varas *et al.* [52] suggested a function for IL-7 *in vivo* for the development of thymic DCs. Using an *in vitro* model (rat fetal thymus organ cultures or FTOCs), the authors showed that IL-7 was able to dramatically increase the numbers of $CD3^-CD4^-CD8^-$ cells that exhibited typical DC markers

(including MHC class II, OX-62, CD11b, CD68, and CD54), morphology and ultrastructural features and were potent stimulators of the allogeneic MLRs. IL-7 induced DC precursor differentiation as the DCs were not actively dividing, precluding expansion of pre-existing cells. Interestingly, the addition of IL-7 to FTOC from severe combined immunodeficient mice also generated large numbers of DC in the absence of thymocyte maturation.

Interleukin-12

In addition to its role in regulation of hematopoiesis, IL-12 has also been shown to contribute to the generation of DCs. *In vivo* studies of DC accumulation in spleen, lymph node, thymus, liver, and tumor tissues indicated that IL-12, alone or in combination with FL, induced the production of DCs which were largely derived from myeloid precursors, while FL induced both myeloid and lymphoid dendropoiesis [53]. The authors suggested that antitumor activity exhibited by IL-12 may, at least in part, be due to this enhanced production of myeloid DCs. Furthermore, IL-12 and FL were also implicated in maturation of DCs. Recently, it was demonstrated that IL-12 acts directly on DCs to induce functional activation through signaling events mediated by the beta1 subunit of the IL-12 receptor [54] that results in increased surface expression of mature class II molecules [55]. MoDCs express the IL12-Rbeta2 while monocytes do not.

Interleukin-13

IL-13 shares a number of properties with IL-4, in spite of the fact that its receptor does not contain the common gamma-chain present in the receptor complex for IL-4 and a number of other cytokines. Piemonti *et al.* [56] first demonstrated the ability of IL-13 to substitute for IL-4, giving rise to DCs with similar phenotypic and functional characteristics following culture with GM-CSF and either IL-4 or IL-13. In addition to cell yield, morphology and membrane phenotype, IL-13- and IL-4-derived DCs exhibited similar capacity for macropinocytosis, stimulating MLRs, and responsiveness to chemokines. Thus the researchers concluded that IL-13 was equivalent to IL-4 for the generation of DCs from PBMCs, suggesting that signaling *via* common cytokine receptor gamma-chain is not essential. Furthermore, DCs generated with IL-13 were shown to be able to effectively induce antigen-specific CTL responses [57]. These data were consistent with studies in which DCs generated from PBMCs of healthy donors as well as breast and melanoma cancer patients using GM-CSF and IL-13, exhibited similar

phenotype, purity and yield to GM-CSF/IL-4 and both populations of DCs were able to effectively present allo- and recall antigens as determined in allogenic and autologous MLRs respectively. In addition, both populations of DCs induced $CD3^+$, $CD8^+$, $CD4^-$ and $CD56^-$ CTLs which were capable of lysing appropriate targets in an antigen-specific manner [58].

Interestingly, not only was IL-13 able to support $CD34^+$ differentiation to DCs in conjunction with GM-CSF, it was in fact, reported to be superior to the "standard" cytokine cocktail of GM-CSF and TNF-alpha *et al.* [58] compared DCs from the PB $CD34^+$ progenitors of G-CSF mobilized healthy donors and breast cancer patients generated with either GM-CSF and IL-13 or GM-CSF and TNF-alpha. One third of the bulk cells cultured with IL-13 were $CD1a^+/CD14^-$ and strongly expressed CD1c, CD40, CD80 and HLA-DR after 15 days in culture. In comparison, although a similar percentage of cells expressed CD1a in response to GM-CSF and TNF-alpha, considerably lower fluorescence intensity was observed, and CD14 was found to be strongly expressed on more than 50% of cells. In addition, IL-13 DCs possessed enhanced capacity to present antigens to autologous lymphocytes and to stimulate allogeneic MLRs compared to TNF-alpha-derived DCs.

Interleukin-15

It has been shown that extended culture for 2-4 weeks with IL-15, as a single agent, has the capacity to induce DC differentiation from human $CD34^+$ hemopoietic precursor cells [59]. Two main populations of cells were distinguished in the cultures, the predominant population (73%-85%) being composed of small to medium-size cells that exhibited a natural killer cell phenotype ($CD56^+$), while the minor population (9%-21%) were comprised mainly of DCs (75%-95% $CD1a^+$). The resultant DCs exhibited the typical morphological, immunocytochemical, phenotypic, and functional characteristics of mature DCs. Flow cytometry analysis performed weekly revealed increasing co-expression of CD1a or CD83 with HLA-DR, CD80, CD86, and IL-2R alpha, beta and gamma subunits. Freshly isolated $CD34^+$ cells did not express IL-2R subunits but acquired expression of the gamma-chain after 2-3 days in culture with IL-15. Induced expression of IL-2Rgamma on $CD34^+$ cells may explain the primary mechanism of IL-15-regulated differentiation of hemopoietic precursor cells. These data suggest that IL-15 may act as a growth and survival factor for lymphoid DCs [59].

Hepatocyte growth factor

Hepatocyte growth factor (HGF) has been known to play a role in the immune response. Ovali *et al.* [60] demonstrated a role for this cytokine in the differentiation of DCs from $CD34^+$ BM cells. $CD34^+$ cells obtained from healthy donors were incubated with various combinations of HGF, GM-CSF and TNF-alpha for 12 days. HGF as a single agent, generated a greater yield of DCs from $CD34^+$ BM cells than GM-CSF, either alone or in combination with TNF-alpha as judged by flow cytometry, morphology and T cell stimulatory capacity. In addition, HGF was also found to potentiate the effect of GM-CSF on DC development while TNF-alpha abrogated the effects. Thus, it appears that HGF can exert a significant effect on the development of DCs from $CD34^+$ BM cells.

CD40 ligand

Although it has been well demonstrated that GM-CSF can act in combination with a number of cytokines to induce DC development from PB monocytes, relatively little work has been done looking at the activity of various cytokine combinations in the absence of GM-CSF. Brossart *et al.* [61] analyzed the effect of FL and CD40 ligand (CD40L) on the development of human DCs in the absence of GM-CSF.

From these studies, it was found that although FL alone is not sufficient to induce DC differentiation, it was able to potentiate this response in conjunction with IL-4, and thus appears to be able to substitute for GM-CSF. These DCs underwent further maturation after stimulation with TNF-alpha or CD40L, as characterized by CD83 expression, upregulation of MHC, cell adhesion, and costimulatory molecules as well as increased ability to stimulate an allogeneic proliferative response. In contrast, ligation of CD40 as a single agent, was able to promote differentiation of adherent blood monocytes into functional DCs in the absence of other cytokines. These cells displayed the phenotypic and functional characteristics of mature DCs and were potent stimulatory cells in priming of MHC class I-restricted CTL responses, although their ability to take up and present soluble protein antigens was reduced. Similarly, human CB and BM $CD34^+$ HPCs expressed CD40 and engagement of the receptor mediated differentiation into DCs [62]. Ligation of CD40 on CB $CD34^+$ HPCs induced cell proliferation and differentiation into a cell population with prominent DC attributes *via* a GM-CSF independent mechanism. These cells displayed strong

expression of the HLA-DR MHC class II molecule and were able to prime allogeneic naive T cells although they lacked detectable CD1a and CD40 expression, features shared by a DC population identified in tonsillar T cell areas.

TRANCE

TRANCE (TNF-related activation-induced cytokine) is a novel member of the TNF superfamily that was cloned during a search for apoptosis regulatory genes [63], and whose expression is restricted to lymphoid-specific organs on T cells [63, 64]. High levels of TRANCE receptor expression are found on mature DCs [65, 66] compared to the low levels found on activated T and B cells, and treatment of DCs with TRANCE results in an enhanced ability to stimulate T cells although it does not increase the T cell stimulatory capacity of DC on a per cell basis and, in fact, does not alter expression of MHC or costimulatory molecules on mature DCs [67]. Instead, TRANCE was found to inhibit apoptosis of mouse BMDCs and MoDC *in vitro*, suggesting a role as a DC survival factor [67]. However, it has also been suggested that TRANCE may modify other aspects of DC function such as modulating the quality of the T cell response, *i.e.* Th1 *versus* Th2. Josien [68] reported that stimulation of antigen-pulsed mature DCs with TRANCE prior to immunization enhanced their adjuvant capacity and improved T cell priming *in vivo* leading to enhanced primary and memory T cell responses. This effect was mediated *via* the DCs as TRANCE did not exert any significant direct effect on the proliferation, activation or survival of T or B cells.

These results were confirmed in other studies where treatment of DCs with TRANCE induced proinflammatory (IL-6, IL-1) as well as T cell growth and differentiation (IL-12, IL-15) cytokines [68]. TRANCE cooperated with CD40L or TNF-alpha to further increase the viability of DCs, suggesting that TNF-related molecules may cooperatively regulate the function and survival of DCs to enhance T cell mediated immune responses. In another report, the TRANCE was able to provide the required costimulation for efficient $CD4^+$ T cells priming during viral infection in the absence of CD40L/CD40 interaction [69].

Macrophage-colony stimulating factor

Kamps *et al.* [70] studied whether it is possible that $CD34^+$ progenitor cells could be induced to differentiate along a DC lineage in response to M-CSF. Indeed, culture with this cytokine was able to induce the generation of $CD83^+$ DC from

$CD34^+$ progenitor cells, in conjunction with TNF-alpha, IL-4, and MGF, although their absolute number was almost threefold lower than the number of $CD83^+$ cells derived from GM-CSF plus TNF-alpha, IL-4, and MGF cultured $CD34^+$ cells. However, as described earlier, there are data suggesting that M-CSF, in conjunction with other soluble factors such as IL-6, can inhibit DC differentiation from $CD34^+$ stem cells. M-CSF was reported to result in the upregulation of the M-CSF receptor, concomitant with a downregulation of GM-CSF receptor levels [47].

Flt3 ligand

FL was first reported to have a mobilizing effect on DCs *in vivo* [71] although recent observations have also reported a capacity to generate DC following *in vivo* adminstration. The adminstration of FL to healthy human volunteers increased the number of circulating DC precusors. Moreover, these circulating DCs were efficient stimulators of T cells *in vitro* [72, 73]. However, the mechanism for the increased DC number is not completely clear and may be a result of increased generation/differentiation from DC precursors, mobilization of preexisting cells, or both. *In vitro*, it has been demonstrated that this cytokine can induce the differentiation of DCs [46, 74]. This factor was able to cooperate with TGF-beta to potentiate *in vitro* development of DCs and allow dendritic cell cluster formation under serum-free conditions [74], while Brasel *et al.* [46] described an FL-dependent BM culture system that generated DCs with diverse phenotypic characteristics. Murine BM cells cultured at high density in recombinant human FL for 9 days, developed into small lymphoid-sized cells, most of which expressed CD11c, CD86, and MHC class II. The $CD11c^+$ population could be divided into 2 populations on the basis of the level of expression of CD11b. These DCs could be activated/matured with IFN-alpha or LPS to express an activated phenotype that included up-regulation of MHC class II, CD1d, CD8alpha, CD80, CD86, and CD40. Interestingly, work by Parajuli *et al.* [75] suggest that FL may act on a distinct population of DCs compared to GM-CSF. The FL-derived DCs also exhibited potent antigen-processing and antigen-presenting capacity. SCF, which exhibits some of the same bioactivities as FL, was unable to replace FL in promoting DC development *in vitro* [46]. Interestingly, FL was found to preferentially induce a DC1 subpopulation which, in turn, elaborated a Th1-biased response, while researchers found that GM-CSF favoured a DC2 subpopulation and a Th2 immune response.

Transforming growth factor-beta

Endogenous TGF-beta is required for the development of both LC and non-LC DC from $CD34^+$ HPCs [76], an effect mediated through proliferation and differentiation of $CD14^+$ and $CD1a^+$ DC precursors respectively. Exogenous TGF-beta polarized the $CD34^+$ HPCs towards LCs by inducing differentiation of $CD14^+$ DC precursors into E-cadherin$^+$, Lag^+CD68^-, and factor XIIIa$^+$ LCs which exhibited functions similar to $CD1a^+$ precursor-derived DC (and contrary to DCs derived from $CD14^+$ precursors in the absence of TGF-beta), in that they did not secrete IL-10 on CD40 ligation or stimulate differentiation of CD40-activated naive B cells. Collectively these results suggested that TGF-beta polarized $CD34^+$ HPC differentiation toward LC. On the other hand, IL-4 induced non-LC DC development independently of TGF-beta [76].

In contrast, Zhang *et al.* [77] found that TGF-beta1 was able to significantly inhibit the generation of $CD11b^{-/dull}CD11c^+$ and $CD11b^{+/hi}CD11c^+$ DC precursors from murine Lin^-c-kit^+ HPCs cultured with GM-CSF, SCF and TNF-alpha. Expression of the cell surface antigen expression of Ia and CD86, as well as RNA levels of MHC class II transactivator and CC-chemokine receptor 7 were suppressed in Lin^-c-kit^+ HPC cultures stimulated with GM-CSF, SCF and TNF-alpha. TGF-beta1 also suppressed development of mature DCs from $CD11b^{+hi}CD11c^+$ DC precursors, but not from $CD11b^{-/dull}CD11c^+$ DC precursors.

CYTOKINE-INDUCED *IN VITRO* MATURATION OF DCS

One aspect of DC biology which has proven to be of particular interest is the dichotomy exhibited in the cellular properties/processes of DCs during maturation that reflect the functional division between immature and mature cells. While immature cells possess very efficient mechanisms for antigen uptake and processing, their presentation capacity is relatively poor, thus making them ideal to function as sentinels in the peripheral tissues with the ability to acquire antigens from invading pathogens. Conversely, mature DCs exhibit reduced antigen uptake capacity while acquiring enhanced presentation ability, thus rendering them ideal to function as cells to stimulate an acquired immune response by efficiently presenting exogenous antigens to immune effector cells. It has now been well documented that DCs arise from multiple lineages and progress through a series of intermediate stages until fully mature, at which time they are capable of optimal antigen presentation and T cell

activation. A wide variety of stimuli are able to induce DC maturation and these include signals from T cells such as CD40L [61, 62], inflammatory cytokines such as TNF-alpha and IL-1, bacterial stimuli such as LPS [6, 78], live gram positive bacteria [79], bacterial DNA and double-stranded RNA [80]. In addition, infection by pathogens such as the influenza virus, can also directly induce the maturation of DCs [81], and recently, additional cytokines have also been reported to induce DC maturation, such as IL-16 and IL-17. Certainly, the ability of DCs to generate an effective immune response has been correlated with their maturation state [81-83].

Interestingly, the reported phenotypic and functional changes associated with the DC maturation process have all been remarkably similar in spite of the wide variety of inducing signals. However, more recently, molecular gene expression analyses using such techniques as DNA array and SAGE (Serial Analysis of Gene Expression) has suggested that, similarities may be superficial and that, in fact, fundamental differences exist between DC populations that have been matured in different ways, which may have functional implications [84, 85].

Interleukin-1

Initial reports indicated that DCs were able to synthesize and secrete IL-1 which acted to stimulate T cells [86, 87]. More recently, it has been found that IL-1 is also able to promote DC maturation [88]. Using the GM-CSF-dependent murine cell line, XS52 as a model system for DC maturation, the authors screened a variety of factors for their ability to mature XS52 cells as judged by enhanced expression of MHC class II, CD40, CD80, CD86 and CD11c molecules, downregulation of CD14 and enhanced ability to stimulate both naive and primed T cells in an antigen-specific manner. IL-1beta (as well as TNF-alpha and CD40 ligation) was found to synergize with IL-4 to induce DC maturation in this system and these matured DCs were potent stimulators of a contact hypersensitivity reaction when conjugated with a hapten. A role for IL-1 was also suggested in work by Williamson *et al.* [89] investigating strategies to convert tolerogenic DCs into immunogenic cells with FL. A combination of FL and IL-1alpha was able to induce a potent active response to orally administered soluble Ag, rather than the normal tolerogenic response. This suggested that IL-1 and other inflammatory signals can act on FL-expanded DCs, and can act to regulate intestinal immune responses [89].

Interleukin-10, interleukin-12

Initiation and development of appropriate immune responses and the optimal elimination of invading pathogens can, in large part, explained by the concept of immune regulation by $CD4^+$ helper T lymphocyte subgroups through the production of mutually exclusive sets of effector cytokines, the so-called Th1 and Th2 cytokines [reviewed in 90]. However, although this is generally accepted immunological dogma, how the immune system makes the Th1/Th2 decision is still not well understood. Recently, it has been hypothesized that Th biasing may be a function of DC derived signalling [91]. DC1 and DC2 cells may initiate either a Th1 or Th2 biased immune response by providing "Signal 3". APCs provide the appropriate signals to T lymphocytes to allow generation of antigen-specific immune responses. These have traditionally included Signal 1 (antigen specific activation by recognition and binding of an appropriate peptide: MHC Class II peptide presented on the surface of the DC to the cognate T cell receptor on the $CD4^+$ T lymphocyte) and Signal 2 (Co-stimulatory signal by recognition and binding of costimulatory molecules on the surface of DCs such as CD40L and CD80/CD86 to their cognate receptors on the surface of T lymphocytes, CD40 and CD28 respectively). However, it now appears that DCs may also provide Signal 3, secretion of either Th1 or Th2 cytokines such as IL-12/IFN-gamma and IL-4/IL-10 respectively, that determine the Th bias of the resultant immune response.

However, although subtypes of DCs produce these different cytokines, it now appears that DCs also respond to stimulation by these same cytokines [92, 93]. The addition of IL-12 results in a dose-dependent maturation of BM-derived DCs generated *in vitro* with GM-CSF and TNF-alpha, as judged by upregulation of costimulatory molecules and enhanced T lymphocyte stimulatory capacity [94]. Similarly, DCs have also been reported to be responsive to IL-10. Consistent with this, De Smedt *et al.* [95] reported that IL-10 induced maturation DCs to a phenotype with impaired ability to produce IL-12 and IFN-gamma (and therefore, elaborated a Th2-biased response). However, in contrast, Cortini *et al.* [96] found that IL-10 actually acted to inhibit DC maturation. By preventing autocrine IL-10 stimulation using neutralizing antibodies, the researchers were able to induce DCs with elevated CD80, 86 and 83 expression, enhanced secretion of Th1 cytokines and enhanced capacity to stimulate T lymphocyte, particularly with a Th1 bias. In any case, these results suggest that DCs are responsive to

autocrine cytokine stimulation that ultimately influence DC maturation and function.

Interleukin-16

Numerous studies have indicated a role for this cytokine in the modulation of DC maturation and activity. IL-16, a natural ligand for the CD4 receptor, has also been found to modulate T lymphocyte function. Some APCs, including macrophages, as well as specific DC populations, have been shown to express functional surface CD4 molecules and these cells have been found to play a critical role in a variety of different immune processes. Recombinant IL-16 was found to upregulate CD25 and CD80 receptor expression on monocyte-derived macrophages but to downregulate CD4 and CD86 surface expression. In MoDCs however, both CD25 and CD83 was upregulated with no change observed in CD4, CD80 or CD86 expression levels. Chemokine receptor gene expression was only modestly and transiently down-regulated following stimulation with IL-16. Based on the ability of IL-16 to up-regulate CD25 and CD83 expression in MoDC, it was suggested that this cytokine might enhance dendritic cell maturation [97].

Interleukin-17

IL-17 is a T cell-derived cytokine that stimulates stromal cells and macrophages to secret proinflammatory cytokines [98]. It has been hypothesized that IL-17 may contribute to allogeneic immune responses during organ and stem cell transplant [99]. To address a possible mechanism by which IL-17 may promote alloreactivity, the influence of IL-17 on the differentiation and function of GM-CSF/IL-4 differentiated BM-derived DCs was investigated. A minor proportion of $CD11c^+$ DCs was found to express the IL-17R, and its ligand was able to promote the maturation of DC progenitors, as demonstrated by increased cell-surface expression of CD11c, costimulatory molecules CD40, CD80, CD86, and MHC class II antigens, as well as enhanced allostimulatory capacity. IL-17 had a lesser effect on the phenotype and function of more fully differentiated myeloid DCs. These findings suggested that the effect of IL-17 may be mediated, at least in part, *via* a maturation-inducing effect on DCs [99].

CD40 ligand

Work by a number of groups have also shown that ligand- or antibody-induced trimerization of CD40 induces DC maturation and enhanced stimulatory ability [100-102]. As such, CD40/CD40L has been widely regarded as a major signaling pathway for DC maturation, which is essential for the initiation of antigen-specific T cell responses and are therefore, considered major players in anti-tumour immunity. Studies using DCs genetically modified *ex vivo* to express CD40L to enhance *in vivo* presentation of tumor antigens to the cellular immune system, show a resultant induction of antitumor immunity and suppression of tumor growth [102].

Recent work, however, has suggested that CD40 ligation may not be sufficient for optimal maturation. Although CD83 has thus been widely accepted as a surrogate marker for a mature DC population, recent work [103] has suggested that IL-12 expression may be a more accurate marker of mature DCs, specifically of functionally active cells that are capable of activating antigen-specific T cells. Based on this criterion, a combination of signaling through CD40 and IFN-gammaR was required to optimally mature DCs. Phenotypically mature DCs could only be induced to produce high levels of IL-12 p70 when provided with both stimulatory signals simultaneously. Interestingly, CD83 expression was necessary, but not sufficient, for optimal production of IL-12, as only a subset of cells expressing high CD80 and CD83 levels synthesized significant amounts of IL-12. DCs matured with both CD40 and IFN-gammaR signals were superior to DCs stimulated with either agent alone in terms of their capacity to activate antigen-specific T cell *in vitro*. In contrast, Kuniyoshi *et al.* [104] found that the production of IL-15 rather than IL-12 was an important factor in the enhanced immunostimulatory ability of CD40-stimulated DCs.

The mechanism by which CD40/CD40L interaction enhances DC function may be through a process that has been termed "DC conditioning", initiated by the recognition of peptide: MHC class II molecules on the surface of DCs by the T cell receptor on CD4 T lymphocytes. This recognition results in the upregulation of CD40L on the surface of CD4 T lymphocytes and subsequent binding to DC CD40. This ligation of CD40 in turn upregulates costimulatory molecules and produces a "conditioned" DC that is capable of efficient interaction with CD8 T lymphocytes expressing appropriate T cell receptors recognizing peptides presented in MHC class I molecules on the surface of the "conditioned DC". In this way, CD4 T cell help may be provided through two distinct pathways, the

more classical concept of cytokine production as well as by CD40L "conditioning" [105].

***Transforming growth factor*-beta**

The immunosuppressive functions of TGF-beta have been well demonstrated, functions that may be mediated, at least in part, by effects on DC generation and maturation (reviewed by Strobl & Knapp [106]). Several studies have shown that TGF-beta1 inhibits *in vitro* activation and maturation of DCs. TGF-beta1 inhibits upregulation of critical T cell costimulatory molecules on the surface of DCs, thereby reducing the antigen-presenting capacity of DCs. Thus, in addition to direct inhibitory effects of TGF-beta1 on effector T lymphocytes, inhibitory effects are also, likely exerted at the level of APCs, which contribute to immunosuppressive effects of TGF-beta.

In contrast, certain subpopulations of immature DCs in nonlymphoid tissues appear to be positively regulated by TGF-beta1 signaling, particularly for development and function of epithelial-associated DC populations. Recent studies have shown that epithelial LCs require TGF-beta1 for development, both *in vitro* and *in vivo*, as well as for enhanced antigen processing and costimulation [106].

***Interferon*-alpha, beta, gamma**

Interferons have been reported to have the capacity to both induce generation [107] and maturation of DCs. The ability of interferons to induce DC maturation has been extensively documented, and has generally been shown to result in increased T lymphocyte stimulatory capacity *via* upregulated expression of specific costimulatory molecules, particularly CD86, and elaboration of various immunomodulatory cytokines [103, 108-112].

Interestingly however, the situation with IFN does not appear to be as simple as reported for other cytokines, since the effect seems to be critically dependent on both the DC and IFN subtype. Ito *et al.* [110] demonstrated that IFN-alpha and -gamma were effective at enhancing viability and inducing maturation of $CD11c^+$ PB DCs as judged by upregulation of costimulatory molecules and induction of a IL-10/Th2-biased response and a IL-12/Th1 biased-response, respectively. In contrast, only IFN-alpha exerted an effect on $CD11c^-$ DC viability, and neither IFN-

alpha, -beta nor -gamma was able induce significant maturation of this subpopulation of DCs. Similar results for DCs generated *in vitro* from monocyte precursors where IFN-alpha induced DC maturation and enhanced function [111-113] have also been reported, although some studies have reported contradictory data. McRae *et al.* [114] recently reported the inhibition of human DCs generated *in vitro* from monocyte precursors by IFN-alpha/beta while Larsen *et al.*, earlier reported IFN-gamma [115] had no effect on DC immunostimulatory function. The basis for these disparate findings is not clear, although the former work dealt primarily with the differentiation rather than maturation of DCs, while the latter study did demonstrate elevated CD86 expression although no enhancement of immunostimulatory capacity was noted. Furthermore, although these studies did not detect any influence of IFN-beta mediated effects, Huang *et al.* [116], researching the mechanism of IFN-beta-induced IL-12 downregulation in multiple sclerosis patients, found that DCs were biased towards a DC2 subtype following exposure to IFN-beta. These cells secreted IL-10, exhibited inhibited IL-12 production, and as expected, elaborated a Th2 mediated T lymphocyte response.

Interestingly however, another effect was observed by Lui *et al.* [117] and Vidalain *et al.* [118] who noted an upregulation of TNF-related apoptosis-inducing ligand on IFN-alpha or -gamma stimulated CD11c PB DCs and IFN-beta-stimulated MoDCs, and a concomittant ability to lyse tumour cells expressing TRAIL receptor, thus indicating a role for IFN matured DCs in direct cytolysis of tumour cells.

CONCLUSION

As reviewed here, an incredibly wide variety of cytokines, under appropriate conditions, have the capacity to differentiate and mature DCs from a variety of precursor populations. There is no doubt that the development of methods to obtain large numbers of DC-like cells have enabled tremendous advances in both our understanding of DC biology and function, as well as our ability to harness these cells to modulate the immune system. Furthermore, the wide array of cytokines, conditions and precursor cells, as well as the properties of the resultant DCs, have made it very clear that *in vitro* generated DCs represent a heterogeneous population, something which parallels the wide variety of DC subpopulations *in vivo*.

However, although the methodology has offered tremendous opportunity in the context of increasing our basic understanding of DC biology, it also suffers from potential drawbacks that may limit its utility. Major among these is the relevance of *in vitro* generated DCs to the *in vivo* condition. *In vitro* derived cells have been and are currently used extensively as models for DC biology, to gain insight into their *in vivo* development and properties, as well as their roles in immune function. However, it is not clear how well the *in vitro* conditions (including cytokines) used to differentiate DCs or the resultant cell populations, actually represent *in vivo* processes. In fact, it is entirely possible that some or most of them have no physiological relevance and do not represent differentiation pathways that occur *in vivo* in spite of the fact that they ultimately generate cells with DC-like properties. Not only would these studies fail to generate meaningful *in vivo* data, they would ultimately be misleading. Therefore, a major challenge confronting the use of *in vitro* models include identifying those conditions that accurately (or adequately) represent *in vivo* processes. Furthermore, in the context of such heterogeneous *in vitro* and *in vivo* DC subpopulations, it is essential to define the specific *in vitro* conditions that reflect specific *in vivo* processes. In this way, it will be possible to effectively use these *in vitro* derived cells to study and understand the properties, functions and roles of specific DCs *in vivo*. Furthermore, by eliminating those conditions that have no *in vivo* relevance, it may be possible to obtain a more unified picture of the processes that lead to the generation and maturation of these cells, something that is currently extremely difficult if not impossible in view of the multitude of cytokines, culture conditions, DC precursors and DC subtypes capable of generating DCs *in vitro*.

However, in terms of clinical application, how well the *in vitro* conditions reflect the *in vivo* scenario is not as relevant as the ability of the DC to fulfill the desired role. It is clear that there is an extensive body of work, both ongoing and completed, aimed at determining the effectiveness of specific cytokines to induce DC subsets. However, the relative capacity of these different cytokines mixtures to induce DC generation and/or maturation, at least in a larger context, remains largely unexplored as does the relative ability of these resultant DC subsets to induce a desired immune effect, either immunogenic or tolerogenic.

In terms of clinical application, the functional/therapeutic characteristics of these DC subsets would undoubtedly have significant consequences on their utility for immunotherapy. Although it is beyond the scope of this review, studies

of *in vitro* DC differentiation and maturation for clinical applications are currently underway. These are largely focused on the induction of antigen-specific immune responses for anti-tumour as well as anti-viral and anti-infectious disease applications, but also include induction of tolerance for the treatment of autoimmune disease, graft-*versus*-host disease and organ rejection. Although the relationship of *in vitro* to *in vivo* DCs may not be relevant in this context, an understanding of the different functions and properties of the various DC subtypes and the effect of maturation state on the quality and quantity of the immune response is essential to optimize the effectiveness of DC-based immunotherapeutic approaches. In this context, both the absolute functionality as well as the relative ability compared to other DC subsets is important. By understanding the functional properties of the different subpopulations and maturational states on the ability of DCs to modulate the immune system, and by subsequently correlating the ability of various cytokines to specifically modulate DC subpopulations and/or DC maturation, we will begin to more fully realize the potential of DC-based immunotherapies to specifically manipulate the immune system.

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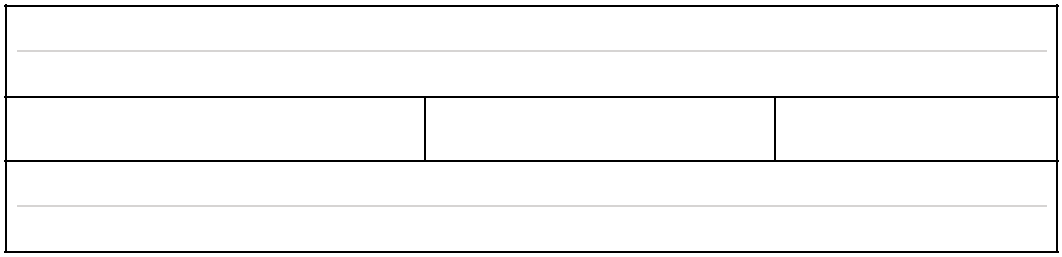
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Figures

Table 1
Culture conditions for generating dendritic cells from various cellular sources

Cellular source	Culture condition	Medium/additive	Reference	Year
Thymic cells	IL-7	RPMI/10% FCS	Varas *et al.* [27]	1998
Bone marrow	SCF, GM-CSF, TNF-α	IMDM/10%FCS	Saraya *et al.*	1996
	SCF, GM-CSF, TNF-α	IMDM/20% FCS	Szabolcs *et al.*	1996
Cord blood	SCF, GM-CSF, TNF-α, CSF, TNF-α	RPMI/10% FCS	Saraya *et al.*	1996
	Flt-3, IL-4, IL-13	RPMI/10% FCS	Rosenzwajg *et al.*	1998
PBSC	GM-CSF, TNF-α	RPMI/1-% FCS	Bernhard *et al.*	1995
	SCF, GM-CSF, IL-4	RPMI/10% FCS	Herbst *et al.*	1997
PBMC	GM-CSF, IL-4	RPMI/10% FCS	Sallusto *et al.* [3]	1994
	GM-CSF, IL-4, TNF-α	RPMI/10% FCS	Zhou *et al.*	1996
	GM-CSF, IL-4, MCM	RPMI/1% plasma	Bender *et al.*	1996
	GM-CSF, IL-7	RPMI/10% FCS	Takashashi *et al.* [26]	1997
	GM-CSF, IL-13	RPMI/10%FCS	Piemonti *et al.* [32]	1995
	IL-2 alone	RPMI1640/10%FCS	Civalloero *et al.* [14]	2000
CD34⁺cells	IL-15 alone	RPMI/15%FCS	Bykovskaja *et al.* [35]	1999
CB CD34⁺ cells	IL-2 alone	RPMI/15 FCS	Bykovskaja *et al.* [13]	1998
BM CD34⁺ cells	GM-CSF+IL-6	X-VIVO10	Bernhard *et al.* [24]	2000
	CD40L	RPMI/10% FCS	Flores-Romo *et al.* [40]	1997
	HGF	IMDM/20 FBS	Ovali *et al.* [36]	2000



Authors

G. M. Zou, Y. K. Tam

Section of Oncology/Hematology, University of Chicago Medical Center, 5841 South Maryland Avenue. MC2115. Chicago. 60637-1470. USA.

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Thioredoxin specifically cross-desensitizes monocytes to MCP-1

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thioredoxin, monocytes, MCP-1.

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