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Cytokine Cross-Talk Between Phagocytic Cells and Lymphocytes: Relevance for Differentiation/Activation of Phagocytic Cells and Regulation of Adaptive Immunity

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Abstract Cytokines represent one of the most important elements in the communication among different cell types. They play an increasingly better understood role in the communication among hematopoietic cells and in particular in the reciprocal regulation of effector cell types of innate or natural resistance (phagocytic cells and Natural Killer (NK) cells) and those of adaptive immunity (T and B lymphocytes). Lymphocytes produce several cytokines with either stimulatory (e.g., colony stimulatory factor) or suppressive (e.g., tumor necrosis factors and interferons) effects on proliferation of early hematopoietic cells. Many of these cytokines, alone or acting in synergistic combinations, also have a differentiation-inducing ability on immature myeloid cells and act as powerful potentiators of the cellular functions of terminally differentiated phagocytic cells. The communication between lymphocytes and phagocytic cells is not unidirectional, as phagocytic cells produce factors that regulate lymphocyte activation. In addition to their role as antigen presenting cells expressing costimulatory accessory molecules and secreting cytokines (e.g., IL-1, IL-6, TNF), phagocytic cells have been recently shown to produce Natural Killer cell Stimulatory Factor (NKSF/IL-12). IL-12 is a heterodimeric cytokine with important modulatory functions on cytotoxicity of NK and T cells, lymphocyte proliferation, lymphokine production, and development of T helper cell subsets. These communications between phagocytic cells and lymphocytes are further regulated by negative and positive feedback mechanisms that contribute to maintain the homeostasis of the system in physiologic conditions and to govern the changes in this equilibrium needed for the response to infectious or other foreign agents.

Key words: lymphocytes, cytokines, macrophages, neutrophils, colony-stimulatory factors, interleukin-12

Lymphocytes, mainly T and NK cells, produce and, in some cases, are the major producers of cytokines that have a major effect on the differentiation and activation of hematopoietic cells. These cytokines include colony stimulatory factors (CSF) such as granulocyte-macrophage CSF (GM-CSF), macrophage CSF (M-CSF), and interleukin 3 (IL-3) as well as other cytokines such as IL-4, interferon-γ (IFN-γ), tumor necrosis factor (TNF), and lymphotoxin (LT) [1]. In the case of pathologic activation or malignant expansion of certain lymphocyte subsets, in patients or in experimental animals, hematopoietic alterations often characterized by neutropenia or anemia are not infrequent. These observations have led to the hypothesis that lymphocytes, T and NK cells, play a major role in the regulation of hematopoietic homeostasis. Although there is no doubt that in pathologic conditions lymphocytes affect the production of both erythroid and myeloid cells in the bone marrow, the physiologic role of T and NK cells is probably a minor one, as normal erythro- and myelo-poiesis are observed in animals deprived of T or NK cells. The observed effects on bone marrow hematopoiesis during in vivo lymphocyte activation could be in part an epiphenomenon due to the fact that many of the lymphocyte-produced cytokines mentioned above have a major physiologic role in activating phagocytic cells and only secondarily in regulating proliferation and differentiation of hematopoietic cells.

The communication between phagocytic cells and lymphocytes is not, however, unidirectional. Phagocytic cells and other cell types of

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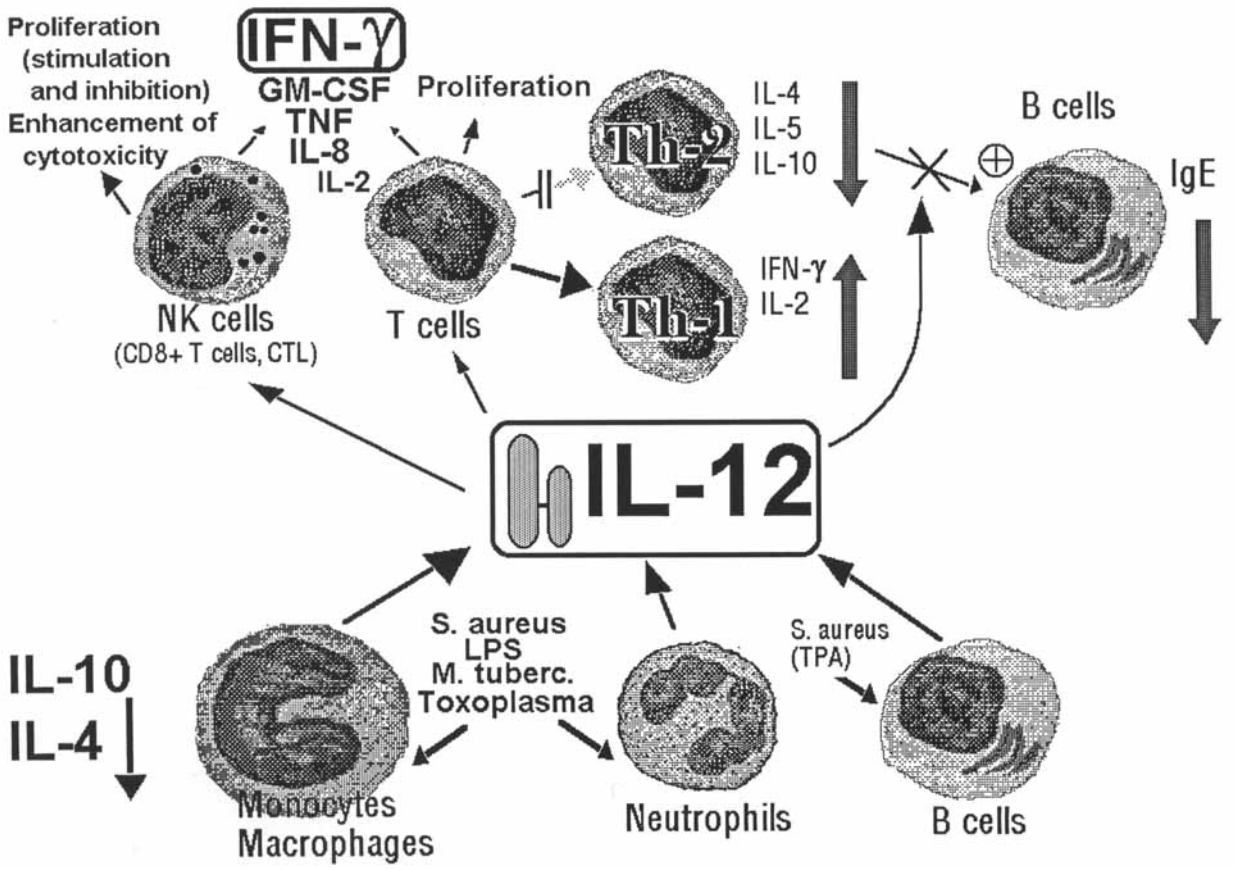

Fig. 1. Schematic representation of the major cytokine pathways between phagocytic cells and lymphocytes.

natural resistance have a major role in regulating the activity of lymphocytes and the immune response. In addition to the well understood role of macrophages as antigen-presenting cells and their ability to produce IL-1, a costimulator of lymphocyte activation, other cytokines produced by phagocytic cells such as IL-6 and TNF have been recognized to play an important role in lymphocyte activation. Natural Killer cell Stimulatory Factor (NKSF or IL-12) is a recently described cytokine that appears to provide an important functional bridge between natural resistance and adaptive immune response [2,3]. NKSF/IL-12 is produced primarily by phagocytic cells and affects T and NK cell functions. It appears, therefore, that a cytokine cross-talk between phagocytic cells and lymphocytes is of central importance in the regulation of both the innate and the immune resistance. In this Prospect, we highlight our work and that of other groups that has led to the identification of different aspects of this cross-talk. Figure 1 schematically depicts some of the principal cytokine interactions between phagocytic cells and lymphocytes.

ROLE OF T AND NK CELLS IN REGULATION OF HEMATOPOIESIS

Lymphocytes represent a small but significant proportion of bone marrow cells from healthy donors. Mature T cells account for a few percent of bone marrow cells and include both CD4 and CD8 subsets [1]. NK cells have been shown to originate and differentiate in the bone marrow, but active mature NK cells are almost entirely absent from the bone marrow of healthy donors [4]. Alteration of T and NK cells in the bone marrow can be quantitative (increased number or change in the proportion of various subsets) or qualitative (activation of the cells). Similarly, NK cells can infiltrate the bone marrow and exist there in an activated state. Although T and NK cells can produce cytokines with either a stimulatory or an inhibitory effect on hematopoiesis, bone marrow failure in one or more lineages is the hematopoietic condition

most often associated with lymphocyte activation. In a classic study by Bagby et al. [5] 234 patients with neutropenia and granulocytic hypoplasia of diverse etiologies were analyzed for evidence of T-cell suppression of granulopoiesis. In approximately one-sixth of the patients suppressive T cells were demonstrated by the observation that elimination of T cells from the bone marrow preparations resulted in increased colony formation, whereas addition of T cells purified from the bone marrow resulted in colony inhibition. Various immunological or nonspecific mechanisms have been proposed to explain the inhibitory effect of lymphocytes on hematopoiesis: 1) the presence of inhibitory lymphocytes may be due to autoimmune mechanisms; 2) the inhibitory lymphocytes may be generated as a reaction to a pathogenic stimulus (e.g., infection or malignancy), and the effect on hematopoietic cells is secondary and non-antigen specific; or 3) the clonal or malignant expansion of a lymphocyte subpopulation with inhibitory activity is responsible for the failure of other hematopoietic cells.

Experimental observations in animals, clinical findings in human patients, and in vitro analysis provided strong evidence that NK cells are involved in the regulation of hematopoiesis [6]. The effector role of NK cells in rejection of parental bone marrow graft in irradiated F1 mice [7] and in suppressing erythropoiesis and phagocytopoiesis in mice infected with lymphocytic choriomeningitis virus (LCMV) [8] demonstrated that in vivo activated NK cells can affect both allogeneic and syngeneic hematopoietic progenitor cells. In vivo depletion of NK cells by treatment of mice with anti–NK cell antibodies demonstrated a differential effect of NK cells on various lineages. NK cell depletion in normal mice increases phagocytopoiesis and decreases erythropoiesis and megakaryocytopoiesis [9,10]. Consistent with these results, in mice receiving myelosuppressive irradiation, depletion of NK cells results in faster recovery of phagocytopoiesis and slower recovery of megakaryocytopoiesis and erythropoiesis [11].

ROLE OF CYTOKINES IN THE EFFECT OF T AND NK CELLS ON HEMATOPOIETIC COLONY FORMATION IN VITRO

Activated lymphocytes or lymphocyte supernatant fluids have been used as a source of CSF in order to obtain hematopoietic colony growth in vitro. However, it was observed that activated T and NK cells or their supernatant fluid are inhibitory for hematopoietic colony formation in vitro in the presence of an exogenous source of CSF. The inhibitory factors produced by activated T cells have been determined to be IFN-γ and LT or TNF, acting synergistically [12]. NK cells also inhibit colony formation by producing IFN-γ and TNF; NK cells, either resting or activated, are induced to produce these cytokines when exposed to autologous or allogeneic hematopoietic progenitor cell preparations [13].

NK cells lyse autologous and allogeneic target cells even in the absence of MHC class I antigens on the target cells. Recently, however, human allospecific NK cell clones have been generated that recognize at least five distinct specificities inherited recessively and controlled by genes linked to the MHC [14]. Because the genetic specificity of these alloreactive NK cells in vitro appears analogous to that of in vivo NK cell–mediated murine hybrid resistance (i.e., the rejection of parental bone marrow in irradiated F1 animals), we [15] tested the ability of human alloreactive NK clones to recognize allogeneic hematopoietic progenitor cells. Alloreactive NK cell clones recognizing specificity 1 significantly and often completely suppressed colony formation by purified peripheral blood hematopoietic progenitor cells from specificity 1 susceptible donors, but had no significant effect on the cells of specificity 1 resistant donors. Activated polyclonal NK cells were less efficient than the NK clones in inhibiting colony formation and had a similar effect on cells from both specificity 1 susceptible and resistant donors. The alloreactive NK clones produced cytokines with a suppressive effect on in vitro hematopoiesis, such as IFN-γ and TNF-α, when exposed to PHA-blasts from specificity 1 susceptible but not resistant donors. However, the mechanism by which alloreactive NK cells inhibit colony formation is more consistent with a direct cytotoxic effect than with the production of inhibitory cytokines because antibodies (anti IFN-γ, TNF-α, and lymphotoxin) which completely blocked the inhibition by polyclonal NK cells had only a minimal effect on the inhibition by the alloreactive clones. Moreover, the alloreactive clones were directly cytolytic in a ^{51}Cr-release assay against enriched preparations of peripheral blood progenitor cells from specificity 1 susceptible donors. These data indicate that the alloreactive NK cells are likely to be the human counterpart of the cells mediating murine hybrid resistance and that these

cells might play clinically important roles in rejection or in graft-versus-leukemia reactions following allogeneic bone marrow transplantation.

EFFECT OF LYMPHOKINES ON THE DIFFERENTIATION OF IMMATURE MYELOID CELLS

CSF produced by T and NK cells induce the proliferation and eventually the differentiation of hematopoietic cells. IFN-γ and TNF/LT, two cytokines which synergistically inhibit myeloid colony formation, are also potent inducers of differentiation of immature myeloid cells [16,17]. The inhibitory effect of these cytokines on proliferation of colony-forming cells is possibly due to their ability to induce early differentiation of the myeloid precursor cells, with loss of proliferative ability. The ability of IFN-γ and TNF/LT, separately or synergistically, to induce differentiation of immature myeloid cells was clearly shown in experiments utilizing myeloid leukemia cells, either cells freshly obtained from patients or established human leukemia cell lines, such as HL-60 or ML-3. The ability of conditioned medium from activated lymphocytes to induce differentiation of myeloid leukemic cells, described in early studies [18], was found to be almost exclusively dependent on the presence of IFN-γ and TNF in the conditioned medium [17].

IFN-γ and/or TNF induce in leukemia myeloid cell lines expression of genes and functions typical of monocyte/macrophages, associated with a decreased proliferative ability of the cells [16,17]. The differentiated cells express surface antigens typical of mature monocytes/macrophages such as CD14, CD11b, CD64, and class II MHC, and acquire phagocytic cell functions such as phagocytosis, the ability to mediate antibody-dependent cytotoxicity, and the ability to respond to external stimuli with an oxidative burst and production of oxygen metabolites. The enzymatic complex responsible for the respiratory burst uses NADPH as a substrate and is referred to as the NADPH oxidase complex [19,20]. We studied the regulation of the expression of four genes encoding proteins essential for NADPH oxidase activity, in order to analyze the ability of the cytokines IFN-γ and TNF to induce functional and phenotypic differentiation of myeloid leukemia cells [21,22]. The factors analyzed were the two chains of the membrane flavocytochrome b capable of directly reducing oxygen to superoxide (the heavy or α chain, gp91-phox, and the light or β chain, p22-phox) as well as the two cytoplasmic proteins required for the cytochrome b activity, p47-phox and p67-phox. Expression of three of the genes encoding these factors, namely gp91-phox, p47-phox, and p67-phox, was induced synergistically by IFN-γ and TNF, whereas the p22-phox gene, encoding the NADPH-binding β chain of cytochrome b, was already expressed in the immature cells and not further induced by the cytokines. The surface markers characteristic of mature cells, the NADPH-oxidase genes, and the phagocytic cell functions were coordinately expressed during in vitro differentiation of the HL-60 and ML3 cells similarly to the orderly regulation of gene expression observed during physiologic differentiation of normal myeloid cells. Cultures of HL-60 and ML-3 cells, in the presence of differentiation inducers, were shown to contain differentiated cells coordinately expressing all the markers analyzed, as well as undifferentiated cells, with a phenotype similar to that of the uninduced cell lines. Figure 2 illustrates a typical experiment illustrating the coordinate expression of cellular functions and gene expression during differentiation. ML3 cells, treated with TNF and IFN-γ, were sorted using flow cytometry (dichlorofluorescein fluorescence) in cells with high and low respiratory burst activity in response to the phorbol diester TPA utilizing the fluorescence of dichlorofluorscein as an intracellular indicator of reactive oxygen metabolites. Accumulation of mRNA for gp91-phox and p47-phox was analyzed by in situ hybridization on the sorted cells; the ML3 cells with the highest respiratory burst activity also showed the highest accumulation of both gp91-phox and p47-phox mRNA.

CYTOKINES PRODUCED BY T AND NK CELLS MODULATE THE ACTIVATION OF PHAGOCYTIC CELLS

Although several cytokines produced by activated lymphocytes affect myeloid cells from the early progenitor cells to mature terminally differentiated cells, their effect on the activation of mature cells, which is responsible for rapid activation of the phagocytic system when T and NK cells are activated in response against infectious microorganisms or other stimuli, is probably the most physiologically relevant. IFN-γ was shown to be the major lymphokine able to activate human macrophage oxidative metabolism and antimicrobial activity [23]. TNF, GM-CSF, and

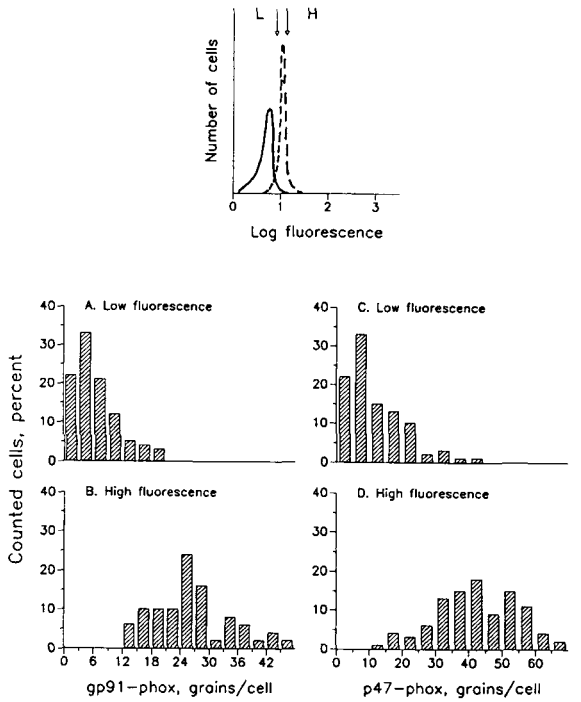

Fig. 2. Distribution of expression of gp91-phox and p47-phox mRNA in ML3 cells sorted for functional oxidative burst capability. ML3 cells were induced with 100 U/ml rTNF and 1,000 U/ml rIFN-γ for 4 days, stained with DCF, activated with TPA for 15 min, and sorted by flow cytometry (**top histogram**) into subpopulations with the 25% lowest (L) and 25% highest (H) fluorescence intensity. The thresholds used for sorting are indicated with arrows on the top histogram. The sorted cells were deposited onto microscope slides and hybridized with RNA probes specific for gp91-phox and p47-phox transcripts. Frequency histograms show distribution of grains/cell of slides hybridized with gp91-phox (**A,B**) and p47-phox (**C,D**) probes in subpopulations of the 25% lowest (A,C) and 25% highest (B,D) DCF fluorescence intensity. Grains/cell over cells hybridized with the sense probes did not exceed 5 grains per cell (not shown). The solid line on the top panel represents the fluorescence distribution of the negative control, and the dotted line represents the distribution of the ML3 cells labeled with DCF.

IL-2 also have activating activity on monocytes/macrophages, in part overlapping with that of IFN-γ. Other products of lymphocytes have down-regulatory effects on macrophage activation and production of cytokines. IL-10, a product of various cell types including B and T cells and monocytes, is a potent inhibitor of monocyte/macrophage functions, including oxidative burst, nitric oxide production, cytotoxicity, and production of cytokines such as TNF, IL-1, and IL-12 [24,25]. IL-4 also has mostly down-regulating activity on monocyte-macrophage function.

Human neutrophils are terminally differentiated cells with a life span in vivo of only a few days. Regulation of the neutrophil system in vivo is mostly regulated by increased production at bone marrow levels as well as migration to the periphery of a preformed bone marrow pool of neutrophils. Lymphocyte-derived cytokines such as GM-CSF and TNF have a physiologic role in vivo in the production and migration, respectively, of neutrophils. Neutrophils have been considered terminally differentiated cells capable of minimal, if any, protein synthesis and with no transcriptional activity. However, studies from our and other groups have clearly shown that neutrophils respond to cytokines such as IFN-γ, primarily, but also GM-CSF and TNF, with de novo transcriptional activity and protein synthesis and expression of new surface markers, production of cytokines such as TNF, IL-8, and, possibly, IL-12, and enhancement of cellular function such as cytotoxicity, respiratory burst, and phagocytosis [26–30]. The first indication of the ability of neutrophils to synthesize new proteins was the ability of IFN-γ to induce on human neutrophils the expression of the high affinity receptor for the Fc fragment of IgG (FcγRI or CD64) [31]. IFN-γ induces both de novo accumulation of mRNA and transcription of the CD64 gene [30,32]. Later, IFN-γ and GM-CSF were shown to enhance neutrophil life span and functions [26,27]. The enhancing effect of IFN-γ on neutrophil respiratory burst was found to be accompanied by a rapid increase in the accumulation of mRNA for gp91-phox, the α chain of the NADPH oxidase cytochrome b [28]. Oligonucleotides antisense to the gp91-phox mRNA prevented the IFN-γ–mediated enhancement of respiratory burst in neutrophils, but not the basal oxidative activity [M.A. Cassatella and G. Trinchieri, unpublished results], suggesting that the IFN-γ effect is in part mediated by up-regulation of the gp91-phox genes, similar to the IFN-γ effect on differentiation of respiratory burst function in immature myeloid leukemia cells [21,22].

PHAGOCYTIC CELLS PRODUCE CYTOKINES WITH REGULATORY ACTIVITY ON LYMPHOCYTE FUNCTION

Phagocytic cells can affect the functions of lymphocytes through different mechanisms. In part, the effects are dependent on cell contact—for example, antigen presentation or expression of costimulatory molecules such as the B7 antigen (i.e., the ligand for the CD28 surface receptor on T cells). Among the cytokines produced by phagocytic cells, several (e.g., IL-1, IL-6, TNF)

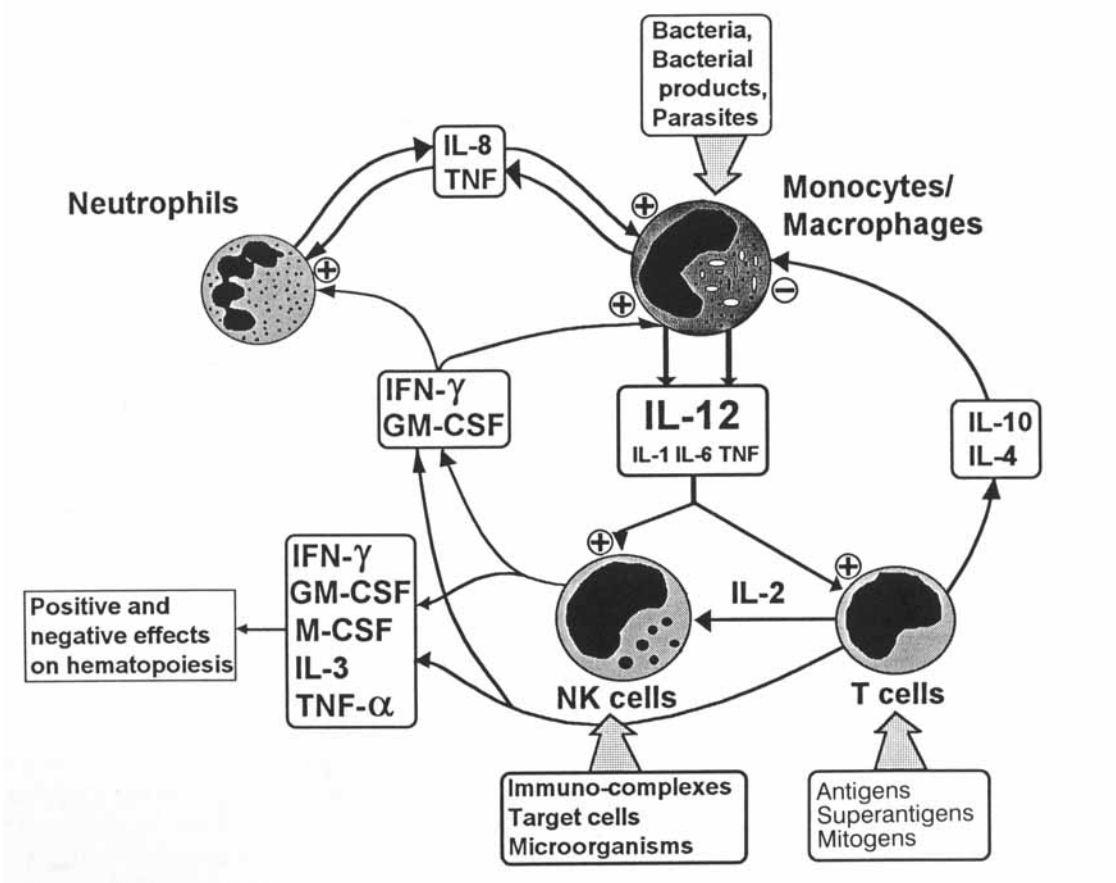

Fig. 3. Schematic representation of the production and biologic functions of Natural Killer cell Stimulatory Factor (NKSF or IL-12).

have a costimulatory effect on T and NK cell activation. However, these cytokines alone have only a minor direct stimulatory effect on lymphocyte proliferation or lymphokine production. The recent identification of NKSF/IL-12 has provided evidence for a new cytokine that may represent the major missing link between the phagocytic cell system and both NK cells and T cells. Figure 3 schematically illustrates the production and major function of NKSF/IL-12.

NKSF/IL-12 is a heterodimeric cytokine of 70 kD formed by a heavy chain of 40 kD (p40) and a light chain of 35 kD (p35) [2]. Although it was originally identified and purified from the supernatant of Epstein Barr–virus-transformed B cell lines, it has been shown that among peripheral blood cells NKSF/IL-12 is predominantly produced by monocytes, with lower production by B cells and other accessory cells [33]. The most powerful inducers of NKSF/IL-12 production are bacteria, bacterial products, and parasites. In addition to the biologically active p70 heterodimer, the cells producing NKSF/IL-12 also secrete a large excess of monomeric p40, a molecule with no demonstrable biological activity [33]. NKSF/IL-12 is active on T lymphocytes and NK cells on which it induces production of lymphokines, enhancement of cytotoxic activity, and mitogenic effects [34–37]. NKSF/IL-12 induces T and NK cells to produce IFN-γ and synergizes with other IFN-γ inducers in this effect. In vitro and in vivo NKSF/IL-12 is required for optimal IFN-γ production [33]. When human lymphocytes are stimulated with antigens in vitro, addition of exogenous NKSF/IL-12 to the culture induces differentiation of T helper cells type 1 (Th1) cells, which produce IL-2 and IFN-γ and favor a delayed type hypersensitivity and cytotoxic T cell generation, whereas neutralization of endogenous NKSF/IL-12 with antibodies favors differentiation of Th2 cells, which produce IL-4, IL-5, IL-6, and IL-10 and facilitate B cell responses and antibody production [38]. IFN-γ, a product of Th1 cells, enhances NKSF/IL-12 production by mononuclear cells, whereas IL-10 and IL-4, prod-

ucts of Th2 cells, efficiently inhibit it [39]. Therefore NKSF/IL-12 appears to be an important inducer of Th1 responses produced by accessory cells during early antigenic stimulation, and its production is regulated by a positive feedback mechanism mediated by Th1 cells through IFN-γ and a negative one by Th2 cells through IL-10 and IL-4. The balance of IL-12 production vs. IL-10 and IL-4 production early during an immune response might therefore be instrumental in determining Th1-type vs. Th2-type immune responses. Because of this potential role of IL-12 during immune responses, the observed impaired ability of HIV seropositive patients to produce NKSF/IL-12 in response to bacterial stimulation (J. Chehimi and G. Trinchieri, unpublished observation) might be a contributing factor to the predominantly Th2 response observed in these patients, which may in part be responsible for their inability to efficiently resist opportunistic infections [40].

NKSF/IL-12, produced by phagocytic cells in response to bacterial or parasitic infection, induces IFN-γ production by T and NK cells, and IFN-γ activates phagocytic cell functions, including their ability to produce NKSF/IL-12. It is therefore evident that NKSF/IL-12 is involved in an effective positive feedback mechanism that induces activation of phagocytic cells during bacterial and parasitic infection [33,41]. Because NK cells are potent producers of IFN-γ in response to NKSF/IL-12 as other stimuli, NKSF/IL-12 is also, at least in part, responsible for the NK cell–mediated, T cell–independent macrophage activation mechanism observed following bacterial infection [42]. A role for NKSF/IL-12 in IFN-γ production and macrophage activation in the absence of T cells has been directly demonstrated in response to Toxoplasma infection [41]. The ability of NKSF/IL-12 to induce IFN-γ production in vivo is, however, not always beneficial. We (M. Wysocka and G. Trinchieri, unpublished data) have observed that NKSF/IL-12 is produced during LPS-induced endotoxic shock and is required for production of IFN-γ, a major factor responsible for shock morbidity and lethality.

CONCLUSIONS

The data briefly summarized in this Prospect clearly indicate that innate resistance, mediated by phagocytic cells and NK cells, and adaptive immunity, mediated by T and B cells, do not act in isolation, but that a reciprocal regulation, mostly through cytokine secretion, regulates the two systems. Some of the cytokines and mechanisms involved in this cross-talk have been extensively studied and are well characterized. The investigation of these last few years has recognized a previously unexpected active role of the neutrophils in these regulatory interactions and has led to the identification and characterization of NKSF/IL-12, a cytokine that appears to provide an essential bridge between innate resistance and adaptive immunity. Pathologic manifestations such as suppression of hematopoiesis or endotoxic shock are probably the opposite extreme effects of the imbalance of the physiologic interaction and cytokine cross-talk between phagocytic cells and lymphocytes. Our knowledge of the cellular and molecular mechanisms involved in these interactions is rapidly progressing, and their complete understanding will allow us to recognize the cause of the pathologic conditions resulting from their imbalance and to plan effective therapeutic intervention.

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REFERENCES

1. Trinchieri G, Murphy M, Perussia B (1987): Crit Rev Oncol Hematol 7:219–265.
2. Kobayashi M, Fitz L, Ryan M, Hewick RM, Clark SC, Chan S, Loudon R, Sherman F, Perussia B, Trinchieri G (1989): J Exp Med 170:827–846.
3. Trinchieri G, Wysocka M, D'Andrea A, Rengaraju M, Aste M, Kubin M, Valiante NM, Chehimi J (1993): Progress in Growth Factor Research 4:355–368.
4. Trinchieri G (1989): Adv Immunol 47:187–376.
5. Bagby GC, Lawrence HJ, Neerhout RC (1983): N Engl J Med 309:1073–1078.
6. Trinchieri G (1992): In Lewis CE, McGee JO (eds): "The Natural Immune System: Natural Killer Cells," Vol 1. Oxford: Oxford University Press, pp 41–65.
7. Cudkowicz G, Hochman PS (1979): Immunol Rev 44:13–41.
8. Randrup-Thomsen A, Pisa P, Bro-Jorgensen K, Kiessling R (1986): J Virol 59:428– 433.
9. Hansson M, Petersson M, Koo GC, Wigzell H, Kiessling R (1988): Eur J Immunol 18:485–488.
10. Pantel K, Nakeff A (1989): Exp Hematol 17:1017–1021.
11. Pantel K, Boertman J, Nakeff A (1990): Radiat Res 122:168–171.
12. Murphy M, Loudon R, Kobayashi M, Trinchieri G (1986): J Exp Med 164:263–279.
13. Degliantoni G, Murphy M, Kobayashi M, Francis M-K, Perussia B, Trinchieri G (1985): J Exp Med 162:1512–1530.
14. Ciccone E, Pende D, Viale O, Di Donato C, Tripodi G, Orengo AM, Guardiola J, Moretta A, Moretta L (1992): J Exp Med 175:709.

15. Bellone G, Valiante NM, Viale O, Ciccone E, Moretta L, Trinchieri G (1993): J Exp Med 177:1117–1125.
16. Perussia B, Dayton ET, Fanning V, Thiagarajan P, Hoxie J, Trinchieri G (1983): J Exp Med 158:2058–2080.
17. Trinchieri G, Kobayashi M, Rosen M, Loudon R, Murphy M, Perussia B (1986): J Exp Med 164:1206–1225.
18. Elias L, Wogenrich F, Wallace J, Longmire J (1980): Leuk Res 4:301–307.
19. Rossi F (1986): Biochim Biophys Acta 853:65–89.
20. Segal AW, Abo A (1993): Trends Biochem Sci 18:43–47.
21. Cassatella MA, Hartman L, Perussia B, Trinchieri G (1989): J Clin Invest 83:1570–1579.
22. Gupta J, Kubin M, Hartman L, Cassatella MA, Trinchieri G (1992): Cancer Res 52:2530–2537.
23. Nathan CF, Murray HW, Wiebe ME, Rubin BY (1983): J Exp Med 158:670–689.
24. Moore KW, Vieira P, Fiorentino DF, Trounstine ML, Khan TA, Mosmann TR (1988): Science 248:1230–1234.
25. Bogdan C, Vodovotz Y, Nathan C (1991): J Exp Med 174:1549–1555.
26. Cassatella MA, Cappelli R, Della Bianca V, Grzeskowiak M, Dusi S, Berton G (1988): Immunology 63:499–506.
27. Perussia B, Kobayashi M, Rossi ME, Anegón I, Trinchieri G (1987): J Immunol 138:765–774.
28. Cassatella MA, Bazzoni F, Flynn RM, Dusi S, Trinchieri G, Rossi F (1990): J Biol Chem 265:20241–20246.
29. Nathan CF (1989): Blood 73:301–306.
30. Cassatella MA, Bazzoni F, Calzetti F, Guasparri I, Trinchieri G (1991): J Biol Chem 266:22079–22082.
31. Perussia B, Dayton E, Lazarus R, Fanning V, Trinchieri G (1983): J Exp Med 158:1092–1113.
32. Cassatella MA, Flynn RM, Aste-Amezaga MA, Bazzoni F, Vicentini F, Trinchieri G (1990): Biochem Biophys Res Commun 170:582–588.
33. D'Andrea A, Rengaraju M, Valiante NM, Chehimi J, Kubin M, Aste-Amezaga M, Chan SH, Kobayashi M, Young D, Nickbarg E, Chizzonite R, Wolf SF, Trinchieri G (1992): J Exp Med 176:1387–1398.
34. Chan SH, Perussia B, Gupta JW, Kobayashi M, Pospísil M, Young HA, Wolf SF, Young D, Clark SC, Trinchieri G (1991): J Exp Med 173:869–879.
35. Gately MK, Desai BB, Wolitzky AG, Quinn PM, Dwyer CM, Podlaski FJ, Familletti PC, Sinigaglia F, Chizzonite R, Gubler U, Stern AS (1991) J Immunol 147:874–882.
36. Gately MK, Wolitzky AG, Quinn PM, Chizzonite R (1992): Cell Immunol 143:127– 142.
37. Perussia B, Chan S, D'Andrea A, Tsuji K, Santoli D, Pospisil M, Young D, Wolf S, Trinchieri G (1992): J Immunol 149:3495–3502.
38. Manetti R, Parronchi P, Giudizi MG, Piccinni M-P, Maggi E, Trinchieri G, Romagnani S (1993): J Exp Med 177:1199–1204.
39. D'Andrea A, Aste M, Valiante NM, Ma X, Kubin M, Trinchieri G (1993): J Exp Med 178:1041–1048.
40. Shearer GM, Clerici M (1992): Chem Immunol 54:21–43.
41. Gazzinelli RT, Hieny S, Wynn TA, Wolf S, Sher A (1993): Proc Nat Acad Sci USA 90:6115–6119.
42. Wherry JC, Schreiber RD, Unanue ER (1991): Infect Immun 59:1709–1715.