Differential Regulation of the Janus Kinase-STAT Pathway and Biologic Function of IL-13 in Primary Human NK and T Cells: A Comparative Study with IL-4

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IL-13, a cytokine similar to IL-4, is a regulator of human B cell and monocyte functions. Biologic effects of IL-13 on primary human NK and T cells have not been well defined. We demonstrate that, in primary NK cells, IL-13, but not IL-4, may induce low levels of IFN-γ secretion. When NK cells were costimulated with IL-13 and IL-2, IL-13 generally resulted in two types of reactivity: IL-13 synergized with IL-2 to stimulate IFN-γ production or it modestly inhibited IL-2-mediated IFN-γ production. In both types of donors, the effect of IL-13 on IL-2-induced IFN-γ production was in marked contrast to the strong inhibition seen with IL-4 in NK cells. Additionally, IL-13 suppresses IL-2-induced NK cytolytic and proliferative activities although less efficiently than IL-4. In T cells, IL-13 inhibits anti-CD3 mAb/IL-2- or PHA-mediated IFN-γ production and enhances cytolytic potential. Furthermore, we demonstrate that IL-13, like IL-4, induces distinct STAT6-DNA binding complexes and tyrosine phosphorylation of STAT6 and Janus kinase 3 (JAK3) in NK and T cells. We observed that Abs directed against unique domains of STAT6 have differential effects on complexes in T cells but not in NK cells, suggesting different STAT6 isoforms. These findings show that IL-13 and IL-4 have the ability to regulate NK and T cell activation and that IL-13 is a potent regulator of STAT6 and JAK3 in these cell types. *The Journal of Immunology,* **1998, 161: 218–227.**

The activation and differentiation of human peripheral blood NK ($CD16^+CD56^+$) and T cells require several extracellular signaling proteins, including IL-2, IL-4, IL-12, and IFNs (1–3). IL-13 is a 13-kDa protein derived from activated T cells and mast cells. Its amino acid sequence is approximated 30% identity to that of IL-4, including two disulfide linkages (4–6). IL-13, like IL-4, has been found to have a critical role for modulating the biologic activity of human B lymphocytes and monocytes. For example, IL-13 can enhance the expression of MHC II and CD23 in monocytes and B cells and induces Ig class switching to IgE in human B cells similar to that observed with IL-4 treatment (4–12). However, the biologic effects of IL-13 on primary NK and T cells have not been well defined.

In human NK cells, while it has been demonstrated that IL-13, unlike IL-4, increases IL-2-induced IFN-γ production (4, 5) and cytolytic activity (4), the IL-13 signaling mechanism in these cells has not been investigated. In human T cells, the biologic effects of IL-13 have not been fully defined although it has been demonstrated that IL-13 is incapable of promoting T cell proliferation (5, 6, 13). In addition, it also has been reported that T cells (5, 13–15), as well as NK and LAK[2] cells (14, 15), lack a functional IL-13R. In contrast, it also has been reported that IL-13, like IL-4, induces IL-4 responsive element DNA protein complexes in human T cells (16) and FcγRI GAS promoter DNA protein binding complexes in fresh human PBL (17). Nevertheless, this limited information suggests that the biologic effects of IL-13 might be distinct when NK and T cells are compared. Therefore, we investigated the signaling and biologic effects of IL-13 on NK and T cells to define the possible different effects of IL-13 on these cell types and to determine whether these effects are distinct from those of IL-4.

The molecular basis for cytokine functional redundancy is thought to occur through shared receptor chains or signaling molecules (18). According to binding assays and functional analysis, it has been demonstrated that IL-13 and IL-4 share common signaling molecule(s) (6, 12, 19–24). In T cells, IL-4Rα conjugates with the common γ-chain (cγ) (25) to form a high affinity IL-4R complex (26, 27). Recently, human and murine IL-13Rα chains, responsible for binding IL-13, were cloned and found capable of associating with the IL-4Rα to form a high affinity IL-13 receptor complex (28–30). Thus, these data suggest that IL-13 might share some biologic effects with IL-4.

The Janus kinase (JAK) STAT pathway is an important cytokine-induced signal transduction pathway that directly transfers signals from cell surface cytokine receptors to the nucleus (31–33). In the case of IL-4, IL-4Rα and cγ are believed to be associated with JAK1 and JAK3, respectively (27, 34). The activation of JAKs leads to phosphorylation of IL-4Rα and recruitment and activation of STAT6 (21, 35–40). STAT6 has been demonstrated to play a critical role in regulating the expression of MHC II, CD23 molecules, and production of IgE in B cells, as well as secretion of IL-4 and IFN-γ in T cells in the STAT6-deficient mouse model (41–43), suggesting that the activation of STAT6 is a key event for IL-4-mediated biologic effects. Therefore, we further investigated whether or not STAT6 and JAK3 were involved in the IL-13-induced signal transduction in NK and T cells.

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[2] Abbreviations used in this paper: LAK, lymphokine activated killer; GAS, IFN-γ activated sequence; JAK, Janus kinase; EMSA, electrophoretic mobility shift assays; PHA, phytohemagglutinin; PE, phycoerythrin; SIE, *sis*-inducible element.

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In this study, we demonstrate that IL-13 is able to differentially modulate specific biologic properties of NK and T cells, including IFN-γ production, cytotoxic activity, and NK cell proliferation, and that these effects are distinctive from those mediated by IL-4. In addition, we found that IL-13 induces distinct activation of STAT6 when primary human NK and T cells are compared. These findings indicate that IL-13 may play an important role in regulating the differentiation and activation of NK and T cells.

Materials and Methods

Medium, Abs, cytokines, and reagents

Cells were cultured in RPMI 1640 (BioWhittaker, Walkersville, MD) and supplemented with 10% FCS (Atlanta Biologic, Atlanta, GA), 100 U/ml penicillin and 100 μg/ml streptomycin (Biofluids, Rockville, MD). Recombinant human IL-2 was provided by Chiron (Emeryville, Ca), and IL-4, IL-6, and IL-13 were purchased from Pepro Tech (Rocky Hill, NJ). IL-12 was provided by Hoffmann-La Roche (Nutley, NJ). Antisera against STAT1α, STAT3, STAT5a, and STAT5b were kindly provided by Dr. Andrew C. Larner (Food and Drug Administration, Center for Biologics Evaluation and Research (CBER), Division of Cytokine Biology, Bethesda, MD). Antisera to STAT4, human STAT6 (residues 828–847, Cat: S-20, denoted as STAT6h1), and mouse STAT6 (residues 280–480, Cat: M-200, designated as STAT6m) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Antiserum to hSTAT6 (residues 787–804, designated as STATh2) was kindly provided by Dr. William J. LaRochelle (Bethesda, MD) (44). Abs against JAK1 and JAK3 were generated as previously described (21, 22). mAb against phosphotyrosine, 4G10, and JAK1 were purchased from Upstate Biotechnology (Lake Placid, NY). A hybridoma producing Ab against CD3 (OKT3) was purchased from American Type Culture Collection (ATCC) (Manassas, VA). Phytohemagglutinin (PHA) was purchased from Murex Diagnostics (Darfort, England). Animal care was provided in accordance with the procedures outlined in the *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health Publication No. 86–23, 1985).

Purification of NK and T cells and cell stimulation

PBMC were separated on Ficoll-Hypaque gradients by leukophoresis packages obtained from healthy donors through the National Institutes of Health blood bank as previously described (45). Leukocyte suspensions were washed in HBSS and resuspended in RPMI 1640 containing 1% heat-inactivated FCS. Adherent cells (monocytes and B cells) were removed by adherence to plastic dishes to nylon wool and via elutriation. Highly enriched populations of $CD3^+$ $CD56^-$ T cells (95–99%) (<2% LGL; large granular lymphocytes) and $CD3^-CD56^+CD16^+$ LGL (>90% pure) (<10% $CD3^+$ T cells, <2% monocytes) were obtained from PBMC by centrifugation of nylon wool-passed and elutriated cells on discontinuous density gradients of percoll (Pharmacia Fine Chemicals, Uppsala, Sweden). The purity of T and NK cells was analyzed by flow cytometry. Activation was monitored by surface activation Ag expression (46). For cytotoxicity and cytokine secretion assays, the cell cultures were treated with 100 U/ml of IL-2, 1 μg of mAb against CD3/10^6 T cells, or 5 μg/ml of PHA.

For EMSA, freshly isolated human NK cells and T cells were immediately washed three times with RPMI 1640 medium to remove contaminating percoll. Next, aliquoted cells were resuspended in RPMI 1640 medium, incubated at 37°C for 30 min in the absence of FCS, and then immediately stimulated with various cytokines at times and concentrations described in the figure legends. Preactivated T cells were obtained by treating freshly isolated T cells with 100 U/ml IL-2 for 72 h. For EMSA, before restimulation, cytokines were removed from the cell culture by washing, and rested in RPMI 1640 medium containing 1% FCS for 24 h at a concentration of 2×10^6 cells/ml.

Cytotoxicity assays and cytokine measurement

Freshly isolated NK and T cells were cultured in the absence or presence of cytokines. The cells harvested from the cultures were used for cytotoxic assays while their supernatants were collected for cytokine analysis. In the cytotoxicity assay, target cells, K562, which were derived from a patient with chronic myelogenous leukemia in blast crisis (47), were labeled with 100 μCi of ^{51}Cr (New England Nuclear, Boston, MA) for 1 h at 37°C and washed. Labeled target cells (5×10^3) and effector cells were cultured together in 200 μl of medium in microtiter plates (Linbro, Hamden, CT) at several effect-to-target ratios (25:1, 12:1, 6:1, 3:1). After 4 h of incubation at 37°C, the supernatant was harvested and counted in a gamma scintillation counter. Cytotoxicity was determined by the amount of ^{51}Cr released from lysed target cells. Three replicates were used per experimental group, and the percentage of specific lysis was calculated according to the formula: lysis = 100 × [(cpm in experimental wells) − (cpm in wells with target cells alone)]/(cpm incorporated in target cells).

IFN-γ production was measured using IFN-γ Elisa Kits (R&D Systems, Minneapolis, MN). All samples were measured in duplicate and plotted against the standard curve and reported as pg/ml. In all assays, the SD of cytokine measurement was less than 25 pg/ml.

Proliferation assay

Freshly isolated human NK or T cells (2×10^5) were cultured in flat-bottom 96-well plates. Each sample was tested in triplicate in 200:l total volume. After 2 to 3 days culture with or without cytokines, wells were pulsed with 1 μCi of methyl[^{3}H]thymidine ([^{3}H]TdR; DuPont NEN, Boston, MA; 6.7 Ci/mmol, ICN Radiochemicals, Costa Mesa, CA) and harvested 4 h later on a Skatron Cell Harvester (Skatron, Sterling, VA). Thymidine incorporation was assessed by liquid scintillation counting.

Flow cytometry analysis

Cells were stained as previously described (48). Cell sorting was performed on either an Epics 750 (Coulter Electronics, Hialeah, FL) or a FACStar (Becton Dickinson, Mountain View, CA). Cells were directly stained using PE or FITC-labeled primary Abs against CD25 or HLA-Dr (Becton Dickinson) or indirectly stained using a primary Ab followed by an isotype-specific FITC-labeled or PE-conjugated secondary or biotinylated primary Ab followed by Streptaviden PE (Becton Dickinson) or Avidium red 670 (Becton Dickinson).

Preparation of nuclear extracts

Nuclear extracts were prepared as described (49). Cells were washed once with cold PBS and once with buffer A (10 mM HEPES, pH 7.9, 10 mM KCl, 1.5 mM $MgCl_2$, 0.5 mM DTT, 1 mM Na-vanadate, 25 mM NaF, 10 mM Na-pyrophosphate, and 25 mM p-nitrophenyl guanidinobenzoate), and lysed in buffer A containing 0.05% NP40. The lysate was placed on ice for 10 min and centrifuged at 4,000 rpm and 4°C for 4 min to remove cytoplasmic proteins. The nuclear proteins in the pellet were extracted in high salt buffer (410 mM KCl, 25% glycerol, and 0.2 mM EDTA in buffer A). Insoluble material was removed by centrifugation at 15,000 rpm for 10 min. Protein concentration was measured with a Bio-Rad (Hercules, CA) protein assay, and samples were stored at −70°C until use.

Electrophoretic mobility shift assays

EMSA was performed as previously described (49). Oligonucleotides were synthesized in DNA/RNA Synthesizer (PE Applied Biosystems, Foster, CA). The double-stranded oligonucleotides containing GAS-like motifs from the human Cϵ regulatory region (5′-AGTCAAGACCTTTTCCCAA GAAATCTATC-3′) (42, 50), and the c-*fos* promoter *sis*-inducible element m67 SIE (5′-AGCTTGTCGACATTTCCCGTAAATCGTCGG-3′) (51, 52) were end-labeled with the Klenow fragment DNA polymerase (New England BioLabs, Beverly, MA) with [α-^{32}P]dATP (3000 Ci/mmol; DuPont) as previously described (49). Labeled DNA probe was further purified from a 12% polyacrylamide gel by excising the corresponding radioactive band after electrophoresis. The purified DNA probe was aliquoted at 15,000 cpm per μl and stored at −20°C until use. For supershift analysis, extracts were incubated with 1 μl antisera to STAT1α, STAT3, STAT5a, STAT5b, and STAT6, while 1 μg anti-STAT4, and 1–2 μl normal rabbit serum were incubated with samples for 2 h on ice, before addition of ^{32}P-labeled DNA probe. Cold competition experiments were performed with unlabeled wild-type probes or Cϵ GAS motif-mutated probes (GTCCCAAGAC). Results were visualized by autoradiography after 1 to 4 days exposure at −70°C.

Immunoprecipitation and Western blotting

Immunoprecipitation and Western blotting were performed as described previously (22). The classified cell lysates were incubated with anti-STAT6 antiserum for 2 h at 4°C. Abs were captured by incubation for 30 min with protein A-Sepharose beads (Pharmacia). Immunoprecipitated protein was washed and eluted by boiling in SDS-sample buffer for 4 min, and then subjected to 7.5% SDS-PAGE under reducing conditions. Proteins were transferred to the polyvinylidene difluoride (PVDF) membrane (Immobilon; Millipore, Bedford, MA; Cat No. 1PVH 00010). The membrane was blotted with 4G10 and then stripped and reblotted with anti-STAT6 antiserum. Proteins were detected with an ECL kit (Amersham, Arlington Heights, IL), according to the manufacturer's instructions.

Table I. *Effect of IL-4 and IL-13 on IFN-γ production and LAK activity in primary NK cells*[a]

Cytokines (per ml)	IFN-γ (pg/ml)		LAK Activity	
	Donor 1	Donor 2	Donor 1	Donor 2
Medium	4	0	24	10
IL-2 (50 U)	24	123	4584	545
IL-4 (10 ng)	5	0	20	1
IL-4 (100 ng)	1	0	51	1
IL-4 (1000 ng)	12	0	32	2
IL-13 (10 ng)	3	3	30	23
IL-13 (100 ng)	28	8	213	30
IL-13 (1000 ng)	57	7	220	24
IL-2 + IL-4 (10)	2	1	121	41
IL-2 + IL-4 (100)	4	0	273	52
IL-2 + IL-4 (1000)	3	0	142	42
IL-2 + IL-13 (10)	293	80	2188	253
IL-2 + IL-13 (100)	690	50	3410	312
IL-2 + IL-13 (1000)	695	60	3488	356

[a] Primary NK cells were incubated with medium, 50 U/ml of IL-2, or 10, 100, or 1000 ng/ml of IL-13, or 10, 100, or 1000 ng/ml of IL-4, or combinations of IL-2 with different doses of IL-13 or IL-4 for 18 h. The cells then were harvested for cytotoxicity assay (denoted as LAK potential), and the supernatants were analyzed for IFN-γ levels (indicated as pg/ml). From nine donors, Donor 1 and Donor 2 were selected as representatives for two distinct NK cell responses from five and four donors, respectively. Lytic potential was calculated at E:T ratio of 25:1.

Table II. *Effect of IL-4 and IL-13 on lytic activity, IFN-γ production, CD25, and HLA-Dr expression in primary T cells*[a]

Stimulation	IFN-γ (pg/ml)	Lytic Activity	CD24 (%)	Dr (%)
Medium	0	7	6	2
IL-4	0	1	9	1
IL-13	0	1	4	2
CD3/IL-2	127	331	35	14
CD3/IL-2 + IL-4	174	6599	38	18
CD3/IL-2 + IL-13	62	1053	50	24
PHA	632	NT[b]	81	40
PHA + IL-4	342	NT	90	26
PHA + IL-13	281	NT	94	36

[a] Primary T cells were cultured with medium, mAb against CD3 plus IL-2, or PHA alone, or in combination of 100 ng/ml of IL-13 or 100 ng/ml IL-4, respectively, with mAb against CD3/IL-2 or PHA for 48 h. The cytokine-treated cells were used in a cytotoxicity assay against K562 (denoted as LAK potential), or measurements of CD25 or Class II (HLA-Dr) expression (indicated % of cells), and IFN-γ secretion (denoted as pg/ml).

Results

IL-13 regulates IFN-γ production in primary NK and T cells

To examine whether IL-13 and IL-4 differentially regulate IFN-γ production in primary NK and T cells, fresh primary NK cells were stimulated with medium, 10, 100, and 1,000 ng/ml of IL-13, or 10, 100, and 1,000 ng/ml of IL-4 in the presence or absence of 50 U/ml of IL-2 for 18 h. Fresh primary T cells were cultured with 100 ng/ml of IL-4 or 100 ng/ml of IL-13 in presence or absence of anti-CD3 mAb (1 μg/10^6 cells)/IL-2 (100 U/ml) or PHA (5 μg/ml) for 48 h. Supernatants from these cultures were harvested for the measurement of IFN-γ by ELISA whereas the cells were collected for cytotoxicity assays (discussed below).

In primary NK cells, IL-13, but not IL-4, could induce low levels of IFN-γ secretion in both representative donors (Table I, donors 1 and 2). When NK cells were costimulated with IL-13 and IL-2, IL-13 generally resulted in two types of reactivity from nine donors tested. Donor 1 is representatives of five of nine donors, in which IL-13 synergized with IL-2 to stimulate IFN-γ production (3- to 27-fold increase) (Table I) and is consistent with previous reports (4, 5). However, donor 2 represents four of nine donors where IL-13 modestly inhibited IFN-γ production (about a twofold reduction) (Table I). Currently, the reasons for variability in IFN-γ production, probably resulting from the differences between NK cell populations in response to IL-13 stimulation, are unknown (i.e., IL-13R or IL-2R expression, signaling molecules, and differentiation states). Nevertheless, in both types of donors, the effect of IL-13 on IFN-γ production was in marked contrast to the strong inhibition seen with IL-4 in NK cells (Table I) since 10 ng/ml of IL-4 was able to effectively inhibit IL-2-induced IFN-γ production.

In primary T cells, IL-4 or IL-13 alone did not induce a detectable level of IFN-γ production (Table II). However, different levels of IFN-γ production were detected in the cultures in which T cells were stimulated with either anti-CD3 mAb/IL-2 or PHA (Table II). IL-13 inhibited both anti-CD3 mAb/IL-2- and PHA-mediated IFN-γ production (approximately 50% reduction) while IL-4 inhibited only PHA-induced IFN-γ production. This inhibition was not seen in anti-CD3/IL-2-treated cells, suggesting that the inhibition might vary under certain T cell activation conditions. However, these data suggest that NK and T cells might express a functional IL-13R, and IL-13 and IL-4 may have different roles in regulating IFN-γ production in these cell types.

IL-13 regulates cytolytic activity in primary NK and T cells

The NK and T cells from the above-mentioned cultures were utilized for cytotoxicity assays. IL-13 itself induced low NK cytolytic activity, while IL-4 had no effect (Table I, donors 1 and 2). In addition, IL-13 had a lower (less than 50%) inhibitory effect on the IL-2-induced NK cytolytic activity (Table I, donors 1 and 2), which was different from the previous report that demonstrated that IL-13 could increase the IL-2-induced LAK activity (4). This difference is probably due to donor-to-donor variations or possibly different sources of IL-13. In contrast, as previously reported (53), IL-4 strongly suppressed IL-2-mediated LAK potential (>90% reduction). These data suggest that both IL-13 and IL-4 have an inhibitory effect on IL-2-induced NK cytolytic activity, but IL-4 is a more potent inhibitor, which is consistent with previous reports that IL-13 and IL-4 have different roles in the regulation of LAK activity (4, 5).

In T cells, IL-13 or IL-4 alone did not induce T cell cytolytic activity (Table II). If T cells were stimulated with anti-CD3 mAb/IL-2 for 48 h, a low level of T cell cytolytic activity was detected. However, when T cells were costimulated with anti-CD3 mAb/IL-2 and either IL-13 or IL-4, both IL-4 and IL-13 increased the cytolytic potential of T cells although IL-4 is much more potent (Table II). These results are consistent with the previous report that IL-4 augmented LAK activity with other T cell mitogens in the absence or presence of IL-2 (54). In contrast, in NK cells, IL-4 and IL-13 inhibited IL-2-mediated LAK activity (Table I). These data suggest that NK and T cells are responsive to IL-13 stimulation and that IL-13, like IL-4, can differentially regulate cytolytic activity in T and NK cells.

IL-13 inhibits IL-2-induced NK cell proliferation, but has no significant proliferative activity in primary T and NK cells

Previous reports have demonstrated that IL-4 can inhibit IL-2-dependent proliferation of human NK and T cells (53, 55), but the effect of IL-13 on NK cell proliferation has not been determined. Freshly isolated NK and T cells were cultured for 60 h in the absence or presence of 10 and 100 U/ml of IL-2, 10 and 100 ng/ml of IL-13, or 10 and 100/ml of IL-4 alone or in combinations of IL-2 with IL-13 or IL-4 as indicated in Figure 1. In NK cells, the

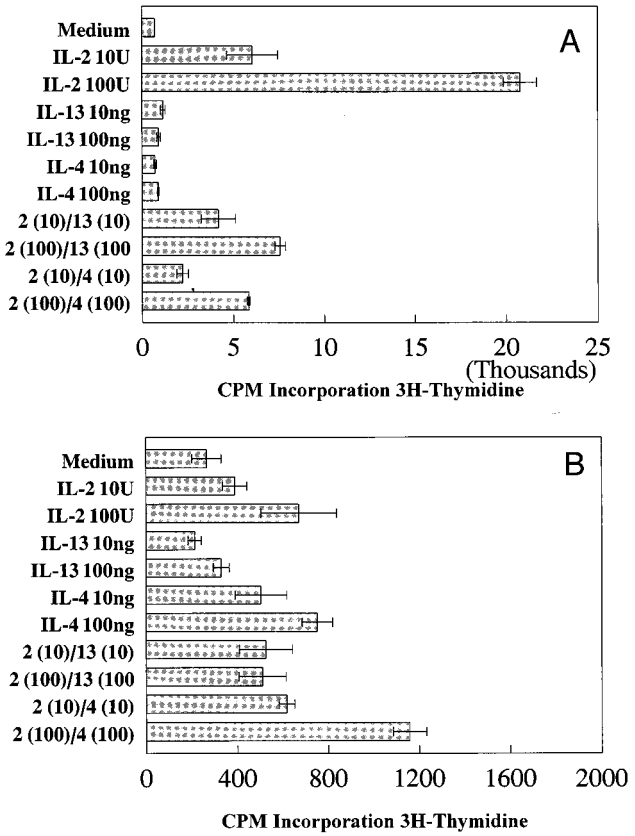

FIGURE 1. IL-13 and IL-4 exhibit a similar inhibitory effect on IL-2-mediated proliferation of NK cells. [^{3}H]thymidine incorporation assays were performed in 2×10^5 primary NK cells (Fig. 1*A*) and T cells (Fig. 1*B*) in 200:l medium. The cells were stimulated with medium, 10 and 100 U/ml IL-2, 10 and 100 ng/ml IL-13, 10 and 100 ng/ml IL-4, respectively, and the combination of IL-2 with either IL-13 or IL-4 as indicated. Results are mean ± SD of triplets. The data was repeated in three experiments in NK cells and in two experiments in T cells.

results of [^{3}H]thymidine incorporation experiments showed that 10 or 100 U/ml of IL-2 significantly induced NK cell proliferation while 10 or 100 ng/ml of IL-13 or IL-4 did not (Fig. 1*A*), suggesting that IL-13, like IL-4, might not be a NK cell mitogen. However, when IL-13 was combined with IL-2, IL-13 inhibited IL-2-induced NK cell proliferation (Fig. 1*A*), similar to that observed with IL-4 (53). These data demonstrate that the inhibitory effect of IL-13 on NK cell proliferation is similar to that of IL-4.

In primary T cells, although IL-2 alone weakly induced T cell proliferation, IL-13 did not have a significant effect on T cell proliferation (Fig. 1*B*). In contrast, IL-4 itself or in combination with IL-2, had a stimulatory (or additive) effect on primary T cell proliferation, consistent with the previous report that IL-4 stimulates T cell growth by an IL-2-independent mechanism (56). The inhibitory effects of IL-4 on IL-2-dependent proliferation of T cells occurred when T cells also were costimulated with anti-CD3 Ab (57). In addition, IL-13 or IL-4 alone did not induce the expression of CD25 and HLA-Dr molecules on the cell surface, while the stimulation of T cells with anti-CD3 mAb/IL-2 or PHA significantly induced CD25 and HLA-Dr expression (Table II). IL-13, but not IL-4, when used in combination with anti-CD3 mAb/IL-2, increased the number of T cells expressing CD25 (from 35% to 50%) and HLA-Dr (from 14% to 24%). In addition, PHA is more effective than anti-CD3-Ab/IL-2 in the induction of CD25 and HLA-Dr expression in T cells, indicating that the PHA-stimulated T cells potentially express more high affinity IL-2R than anti-CD3 Ab/IL-2 stimulated ones, suggesting that IL-13 might expand the pool of IL-2 responding cells (Table II).

IL-13, like IL-4, induces distinct Cϵ DNA-protein binding complexes in fresh primary human NK and T cells

Transcription factor STAT6 is one of the key elements in the transduction of IL-4-mediated signaling (41–43). To determine whether NK and T cells were responsive to IL-13 stimulation resulting in STAT6 activation, nuclear extracts were prepared from fresh primary T and NK cells stimulated with IL-13, IL-4 (positive control), IL-6, and IL-12 (negative control) for 15 min at 37°C. Using a Cϵ STAT6 DNA-binding element as an oligonucleotide probe (42, 50), EMSA analysis revealed that IL-13 and IL-4 induced two similar Cϵ DNA-protein complexes in fresh primary NK cells, which were designated as complexes B and C, according to their electrophoretic mobility (Fig. 2*A*, *lanes 3* and *4*). We also observed a slow migrating band (designated as complex A) induced by IL-13 and IL-4 in fresh primary T cells (*lanes 8* and *9*) that was not seen in NK cells. In addition, the complex C appeared constitutively at low levels in primary T cells in some donors (Fig. 2*A*, and data not shown). In contrast, IL-6 and IL-12 did not induce any specific DNA protein complex with this oligonucleotide probe (*lanes 2*, *5*, *7*, and *10*). These results revealed several interesting findings. First, the ability of IL-13 to induce Cϵ GAS DNA-protein

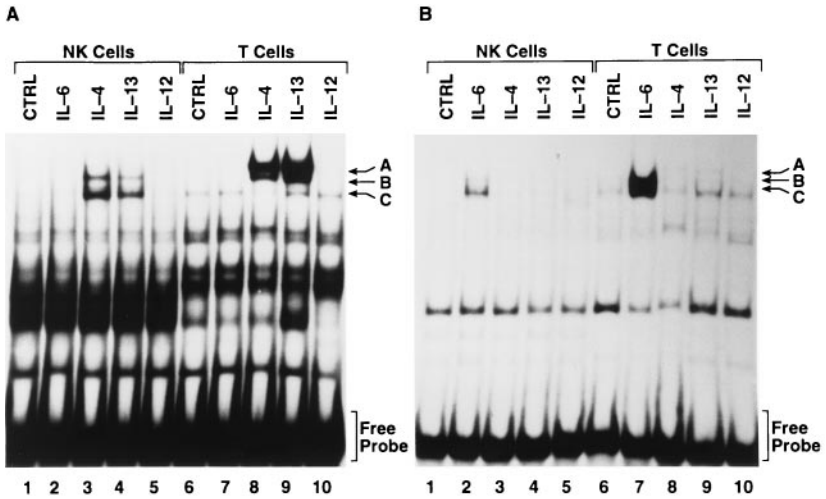

FIGURE 2. IL-13 displays distinct Cϵ DNA-protein-binding complexes in primary human NK and T cells. *A*, Primary NK (*lanes 1–5*) and T cells (*lanes 6–10*) were stimulated by medium as control (CTRL), IL-6 (100 ng/ml), IL-4 (100 ng/ml), IL-13 (100 ng/ml), or IL-12 (100 U/ml) for 15 min. Ten micrograms of nuclear proteins prepared from each sample were used for EMSA using ^{32}P-labeled Cϵ GAS oligonucleotide probe. *B*, The same nuclear extracts described in (*A*) were used for EMSA using a ^{32}P-labeled SIE GAS oligonucleotide probe. Arrows A, B, and C denote DNA-protein complexes A, B, and C. Bracket indicates free ^{32}P-labeled oligonucleotide probe. Similar data was obtained from cells obtained from four other donors.

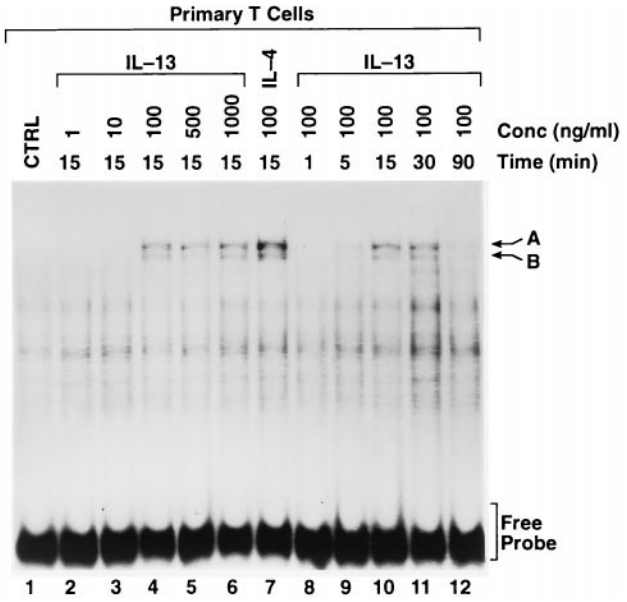

FIGURE 3. Dose and time-dependence of IL-13-induced Cϵ DNA-protein-binding complexes in primary T cells. Primary T cells were stimulated with medium as control (CTRL), 1, 10, 100, 500, 1,000 ng/ml of IL-13 for 15 min (*lanes 1–6*), 100 ng/ml IL-4 (*lane 7*), or with 100 ng/ml IL-13 for 1, 5, 15, 30, and 90 min (*lanes 8–12*). Ten micrograms of nuclear proteins were used for EMSA using a ^{32}P-labeled Cϵ GAS oligonucleotide probe. Arrows A and B denote DNA-protein complexes A and B. Bracket indicates a free ^{32}P-labeled oligonucleotide probe.

complexes further suggests that primary human NK and T cells express functional IL-13R. Second, IL-13 and IL-4 induce similar binding complexes in NK or T cells. Third, distinct DNA-protein binding complexes are induced by IL-13 and IL-4, when comparing both cell types.

It is well established that STAT proteins show preferential binding affinity to different DNA-recognition sequences (31, 58). To explore the possibility that IL-13 and IL-4 induce distinct DNA-binding complexes when NK and T cells are compared, we analyzed these cells using a SIE GAS-like oligonucleotide probe that preferentially binds STAT1α, STAT3, and STAT4 (induced by IL-6 and IL-12, respectively) (49, 52, 59, 60). IL-13 and IL-4 did not induce DNA-protein complex formation with its oligonucleotide probe (Fig. 2*B*, *lanes 3, 4, 7,* and *8*). In contrast, IL-6 induced three SIE GAS DNA-protein-binding complexes with similar mobility (*lanes 2* and *7*) when comparing NK and T cells, while IL-12 induces two unique bands upon prolonged exposure (data not shown). These results indicate that different GAS-like element DNA probes (ie, Cϵ or SIE) can selectively bind activated STAT proteins as evidenced by the unique binding patterns. Moreover, these data also suggest that IL-13 and IL-4 might not significantly induce the activation of STAT1α and STAT3 proteins in primary NK and T cells.

Dose and time-dependence of IL-13-induced Cϵ DNA-protein-binding complexes

To further characterize IL-13 activation of STAT6 in NK and T cells, time-course and dose-dependence assays were performed. Cells were stimulated with 1, 10, 100, 500, or 1,000 ng/ml of IL-13 for 15 min or with 100 ng/ml of IL-13 for 1, 5, 15, 30, and 90 min for EMSA assay, or with 100 ng/ml of IL-4 as a positive control (Fig. 3). IL-13 (10 ng/ml) very weakly induced Cϵ DNA-protein-binding complexes while 100 (8 nM), 500, and 1000 ng/ml of IL-13 induced two Cϵ DNA-protein-binding complexes (designated as complexes A and B) (Fig. 3, *lanes 4, 5,* and *6*). Peak induction of Cϵ DNA-protein-binding complexes was found to occur between 15 and 30 min (*lanes 10* and *11*). These results are similar to those observed with IL-4 induction of IL-4NAF-binding complexes (35). Lastly, 100 ng/ml of IL-4 induced the same complexes that were induced by IL-13 (Fig. 3, *lane 7*).

IL-13-induced Cϵ DNA-protein complexes contain STAT6 protein(s)

To identify the protein components in IL-13- and IL-4-induced Cϵ DNA-binding complexes, supershift analysis with STAT6-specific Abs was performed (Fig. 4, *A* and *B*). Three anti-STAT6 antisera were tested, which recognize distinct STAT6 sites (as described in *Materials and Methods*). In the IL-13-treated nuclear extracts, Ab prepared against amino acids 827–847 (designated STAT6h1) effectively blocked and supershifted both complexes A and B (Fig. 4*A*, *lane 3*). The other Ab recognizing amino acids 787–804 (designated STATh2) blocked and supershifted only complex A (Fig. 4*A*, *lane 5*). In contrast, antisera against STAT3 and STAT6m (*lanes 2* and *4*) had no effect on these DNA-protein complexes. These results indicated that complexes A and B contain STAT6 protein(s). Whether Ab prepared against amino acids 787–804 is less efficient or incapable of recognizing possible STAT6 isoforms or other associated protein involvement is unknown. Similar supershift results also were obtained in nuclear extracts prepared from IL-4-treated T cells (Fig. 4*B*). Complexes A and B induced by IL-4 contained STAT6 proteins, but not STAT3 and STAT4 (Fig. 4*B*, *lanes 2, 3,* and *5*), or STAT5a and STAT5b (data not shown). Western STAT6 supershift results were obtained with NK cells (data not shown). These data demonstrated that IL-13 is able to directly activate STAT6 in fresh primary T and NK cells, analogous to IL-4.

IL-13 induces different patterns of Cϵ DNA-protein-binding complexes in preactivated primary T cells vs fresh primary T cells

The activation of primary T cells for several days by various stimuli, such as IL-2, anti-CD3 Ab, or PHA, leads to morphologic and functional cell changes. To determine whether preactivated T cells, stimulated with PHA/IL-2 for 3 days (described in *Materials and Methods*), are distinct from the freshly isolated primary T cells stimulated with IL-4 or IL-13, STAT6 activation was measured by EMSA, and supershift analysis was performed (Fig. 5). In both primary (*lane 1–3*) and preactivated T cells (*lanes 4–12*) obtained from the same donor, IL-4 and IL-13 induced complex A and B in primary T cells (Fig. 5, *lanes 2* and *3*) (see also Figs. 2 and 3) while significantly inducing three Cϵ DNA-protein-binding complexes (designated as A, B, and C) in the preactivated T cells (Fig. 5, *lanes 5* and *6*). Similar results were observed in three other donors (data not shown). IL-2 did not induce any DNA-protein complex in this donor's cells, but a single band, more slowly migrating than the bands induced by IL-13 or IL-4, was seen with cells obtained from other donors (data not shown).

The supershift analysis showed that both complex A and complex B were blocked with anti-STAT6h1 antiserum (Fig. 5, *lane 7*) while only complex A was blocked by anti-STAT6h2 antiserum for IL-13- and IL-4-treated cells (data not shown). This result is consistent with results described in Figure 4. However, complex C was neither blocked nor supershifted by anti-STAT1α, -STAT3, -STAT4, or -STAT5a/b (Fig. 5, *lanes 8, 9,* and data not shown). To elucidate the identity of complex C, specific and nonspecific cold oligo competition assays within EMSA were performed. Complex C, as well as complexes A

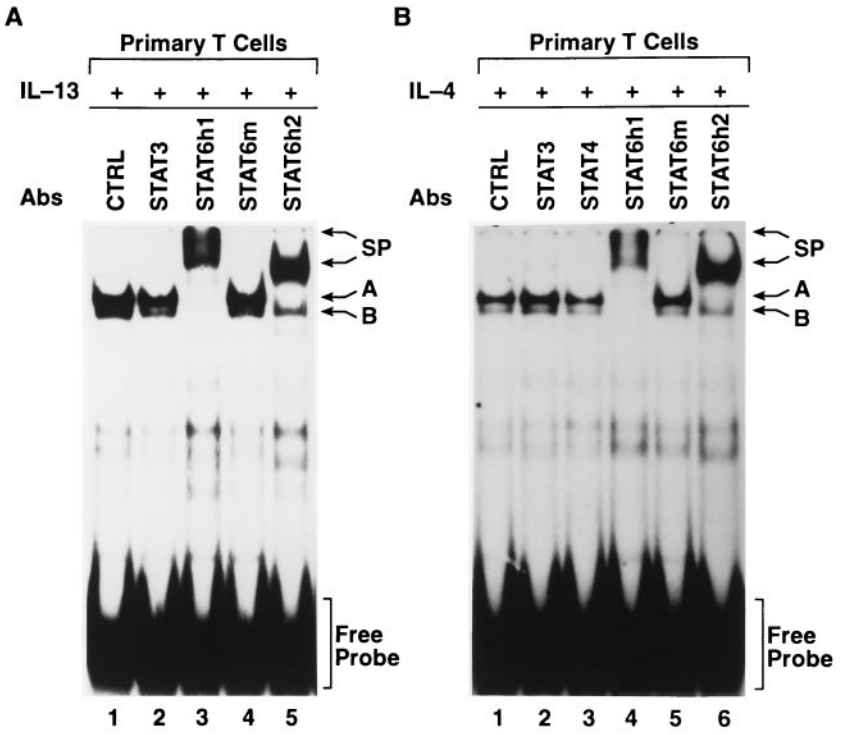

FIGURE 4. IL-13-induced Cϵ DNA-protein complexes contain STAT6 protein(s). *A*, Ten micrograms of nuclear proteins prepared from fresh primary T cells treated by IL-13 (as described in Fig. 2) was used for EMSA supershift analysis using a ^{32}P-labeled Cϵ GAS oligonucleotide probe with antisera against STAT3, STAT6h1 (*lane 3*), STAT6m (*lane 4*), and STAT6h2 (*lane 5*). *B*, Supershift analysis was performed using nuclear proteins prepared from IL-4-treated fresh primary T cells (as described in Fig. 2) with antisera against STAT3, STAT4, STAT6h1, STAT6m (*lane 5*), and STAT6h1 (*lane 6*). Arrows A and B denote DNA-protein complexes A and B while SP indicates supershifted complexes. Bracket indicates free ^{32}P-labeled oligonucleotide probe.

and B, was completely competed with 1 ng of specific cold Cϵ oligonucleotides but not with 1 ng SIE or mutated oligos, suggesting that complex C is a specific one. Therefore, complex C may be specific for preactivated primary T cells. These data suggest that IL-13 and IL-4 might induce different patterns of STAT6 activation when comparing preactivated and fresh primary T cells and further suggest that STAT6 might be differentially utilized in various T cell types.

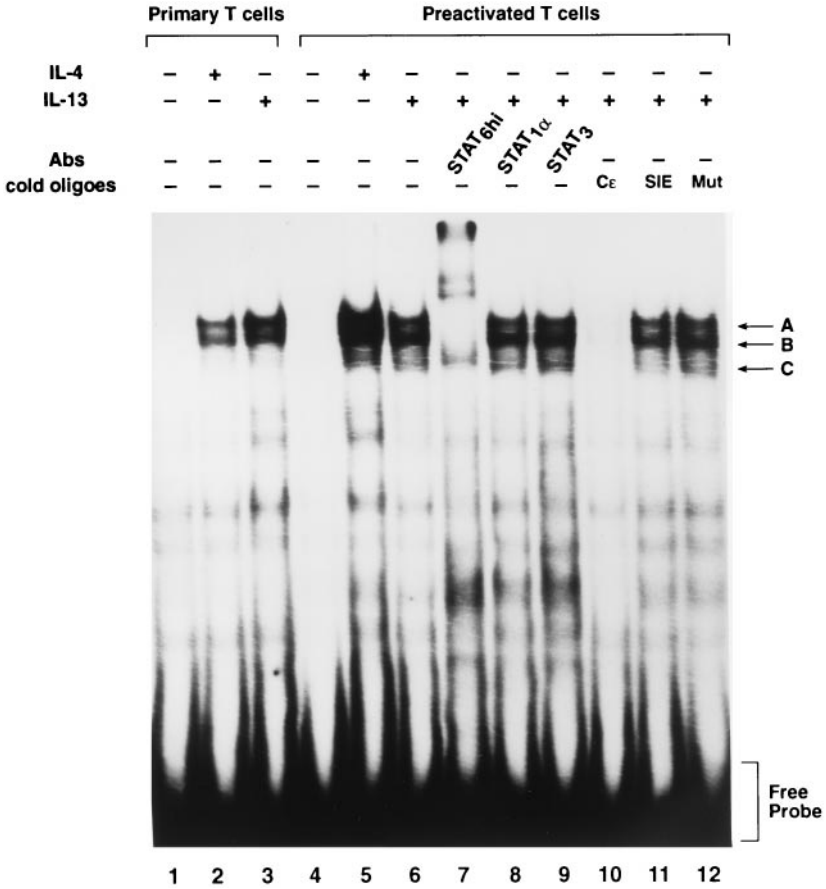

FIGURE 5. IL-13 induces different patterns of Cϵ DNA-protein-binding complexes with primary freshly isolated (*lanes 1–3*) and preactivated primary T cells (*lanes 4–12*). Nuclear proteins (10 μg) were prepared from primary and preactivated T cells that had been treated with medium (*lanes 1* and *4*), IL-4 (*lanes 2* and *5*), or IL-13 (*lanes 3* and *6–12*), as described in Figure 2. STAT activation was examined by EMSA using a ^{32}P-labeled Cϵ GAS oligonucleotide probe. Cold oligonucleotide competition assays were performed with 10 μg of nuclear proteins prepared from IL-13-treated (*lanes 6–12*) preactivated T cells with antisera against STAT6h1 (*lane 7*), STAT1α (*lane 8*), STAT3 (*lane 9*), cold Cϵ- (*lane 10*), cold SIE- (*lane 11*), or mutated Cϵ oligo (*lane 12*). Arrow SP indicates supershifted complexes. Bracket indicates free ^{32}P-labeled oligonucleotide probe.

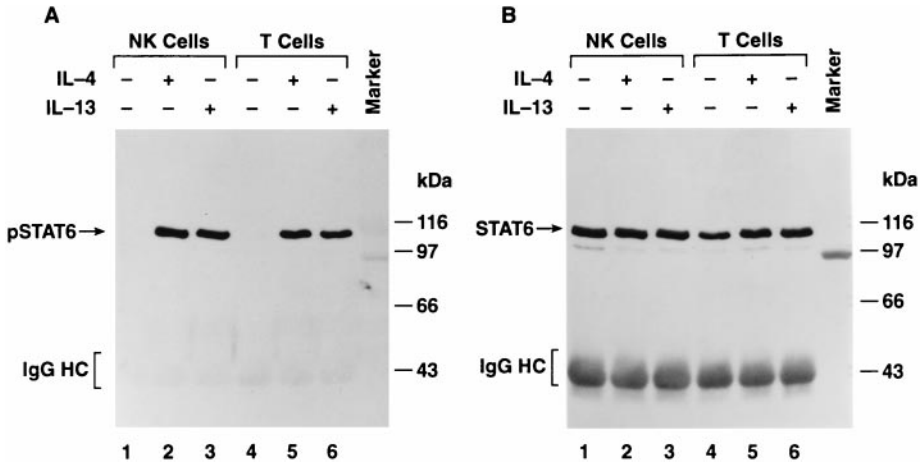

FIGURE 6. IL-13 induces the tyrosine phosphorylation of STAT6 in primary NK and T cells. *A*, Cell lysates from 6×10^7 NK (*lanes 1–3*) or T cells (*lanes 4–6*) were stimulated with medium as control (CTRL), IL-4 or IL-13 (as described in Fig. 2), and immunoprecipitated with anti-STAT6 antisera. Immunoprecipitated protein was blotted with the anti-phophotyrosine Ab, 4G10. Arrow denotes phosphorylated STAT6, pSTAT6. *B*, The blot was stripped and reprobed with anti-STAT6 antiserum. Arrow denotes STAT6 proteins. Bracket indicates Ig heavy chain (IgG HC). Molecular size markers are indicated on the right side.

IL-13 induces activation of STAT6 via tyrosine phosphorylation of STAT6 and JAK3

To determine whether tyrosine phosphorylation of STAT6 occurs following IL-13-treatment in NK and T cells, we analyzed lysates of stimulated cells with anti-STAT6 antiserum via immunoprecipitation (Fig. 6). Anti-phosphotyrosine Western blotting revealed that STAT6 was phosphorylated following IL-13 stimulation in NK cells (*lane 3*) and T cells (*lane 6*) in a manner analogous to IL-4-treated cells (*lanes 2* and *5*). This blot was then stripped and reprobed with anti-STAT6 antiserum to verify equal amounts of loaded protein (Fig. 6*B*). A small fragment migrating with an apparent molecular mass of 100 kDa also was recognized by the anti-STAT6 antiserum although it was not detected with anti-phosphotyrosine Ab (Fig. 6*B*). Whether this protein represents an isoform of STAT6 or a degraded product is currently being investigated. In any case, these results demonstrate that IL-13, as well as IL-4, induce STAT6 tyrosine phosphorylation in both cell types.

To determine whether IL-13, like IL-4, is able to induce the tyrosine phosphorylation of JAK3 and JAK1 in primary T cells, the immunoprecipitation assays of JAK3 and JAK1 were performed with extracts from the preactivated primary T cells that have been stimulated with medium, 100 ng/ml of IL-13, and 100 ng/ml of IL-4 for 15 min respectively. The results showed that IL-13 induces the tyrosine phosphorylation of JAK3 although the level of IL-13-induced tyrosine phosphorylation of JAK3 was lower than induced by IL-4 (Fig. 7). However, in comparison with JAK3, IL-13 very weakly induced the tyrosine phosphorylation of JAK1 (data not shown). However, these results suggest that IL-13 induced the activation of STAT6, due to the activation of JAK3.

Discussion

In this report, we demonstrate that IL-13 and IL-4 differentially regulate specific functional activities of NK and T cells. Moreover, we provide evidence that IL-13, similar to IL-4, induces the activation of STAT6 and JAK3 in fresh primary T and NK cells. This conclusion is based on EMSA, supershift analysis, immunoprecipitation, and Western blot analysis. In addition, we found that IL-13, as well as IL-4, induced distinct and overlapping patterns of Cϵ DNA-protein-binding complexes when comparing primary NK cells and T cells. These results suggest that STAT6 may be differentially utilized in a cell-dependent fashion and that IL-13 may play an important role in the regulation of NK and T cells.

We have observed that IL-13, but not IL-4, weakly induced IFN-γ production in NK cells and either synergized with IL-2 or weakly inhibited (with 30–50% reduction) IL-2-mediated IFN-γ production. This result likely reflects the variability in NK cells seen from donor to donor. However, in both situations, the IL-13 effect was in marked contrast to the strong inhibition (with more than 90% reduction) seen with IL-4 in NK cells (Table I). The different effects of IL-13 and IL-4 on IFN-γ production in NK cells also were confirmed by analyzing the effects of IL-4 and IL-13 pretreatment for the first 24 h on IL-2-mediated IFN-γ production (data not shown). These results demonstrate that IL-13 and IL-4 might have a different role in regulating IFN-γ production in NK cells. However, in T cells, IL-13 and IL-4 similarly inhibit PHA-induced IFN-γ secretion. This is in contrast to the effect of IL-4 on NK cells where it acts as a strong inhibitor of IFN-γ production. Furthermore, in NK cells, IL-13 weakly and IL-4 strongly inhibit the IL-2-induced cytolytic activities whereas, in T cells, both IL-13 and IL-4 enhance CD3/IL-2-mediated LAK potential. These data also suggest that T cells express functional IL-13R and IL-13 might share similar biologic effects with IL-4 in NK and T cells. A previous report has demonstrated that IL-13 was able to increase IL-2-mediated LAK activity, while our data demonstrated IL-13 weakly inhibits this activity. The discrepancy in these findings may be due to donor differences or the different sources of IL-13 used

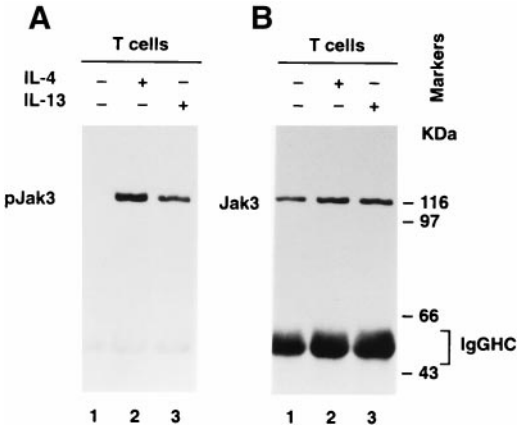

FIGURE 7. The immunoprecipitation assays against JAK3 and JAK1 were performed in the preactivated primary 6×10^7 T cells that have been stimulated with medium (CTL), 100 ng/ml of IL-4, and 100 ng/ml of IL-13 for 15 min, respectively. *A*, Immunoprecipitated protein was blotted by anti-phosphotyrosine Ab, 4G10. The arrow denotes phosphorylated JAK3, pJAK3. *B*, The blot was stripped and reprobed with anti-JAK3 antiserum. Arrow denotes JAK3 proteins. Bracket indicates Ig heavy chain (IgGHC). Molecular markers are indicated on the right.

in these studies. However, it is consistent with a previous report that IL-13 and IL-4 have a different role in the regulation of LAK activity. Collectively, this evidence further elucidates that NK and T cells are functionally regulated by IL-13 and that its effects on NK and T cells are unique and overlapping compared with IL-4.

Previous studies have suggested that the functional IL-13R are not expressed on T cells (5, 13–15), LAK (15), and NK cells (14). However, IL-13, like IL-4, has been demonstrated to induce IL-4 responsive element DNA-protein complexes in primary human T cells (16) or FcγRI-binding DNA-protein complexes in fresh human PBL (17). Thus, a potential question arises as to whether or not the cells utilized in the experiments were contaminated by monocytes or B cells. However, in the data presented in this manuscript, the primary NK and T cells did not contain a significant level of contaminating monocytes and B cells, based on fluorescent staining for phenotype determination. In addition, we have observed different patterns of STAT6 activation between NK and T cells, which also could not be explained by the possibility of monocyte and B cell contamination in these cell preparations. Moreover, IL-13 is able to modulate the NK and T cell biologic activities as described above. Thus, our data provide evidence that IL-13 may regulate primary T cells and thereby substitute for IL-4, perhaps explaining why impairment in Th2 development seen in STAT6$^{-/-}$ mice is more pronounced than observed in IL-4-deficient mice (43). Therefore, in this manuscript, we have provided important observations that offer insight into the biology of IL-13 and on the expression and function of IL-13R on human T and NK cells.

Data from Table I would suggest that IL-13 is not a significant inducer of IFN-γ production or LAK activity for NK cells, but perhaps acts as a co-regulatory agent when combined with IL-2. Indeed, many factors regarding NK cell responsiveness to IL-2, such as levels and types of IL-2R expression, may all determine differential biologic outcomes following IL-13 stimulation. In the same donor, what appears to be clear is that IL-13 or IL-4 can have different or opposing effects on IL-2-induced IFN-γ production. Whether these effects are due to differences in unique signal transduction pathways rather than being dependent on the levels of IL-2R expression alone is not readily apparent and requires future study.

It is known that the primary NK and T cells express different IL-2R complexes (61). Therefore, these differences may determine the type of IL-13 effect seen in NK cells. In addition, the receptor components for IL-13R complex and its state of expression on NK and T cells is not clear. On the other hand, IL-13 and IL-4 have overlapping and distinct biologic effects on both NK and T cells. Here, we have demonstrated that IL-13 and IL-4 are similarly able to induce distinct Cϵ GAS DNA-protein-binding complexes between NK and T cells, but they only partially share similar biologic effects on IL-2-induced proliferation and LAK activity, but not IFN-γ production in NK cells. These data suggest that the differential effects of IL-13 on NK and T cells might result from cell-dependent signaling pathways, differentiation status, and unique cytokine-signaling pathways.

While the signaling pathway responsible for mediating IL-13 responses is not well established in NK and T cells, the IL-4Rα probably serves as the signaling transducing subunit responsible for activation of STAT6, 4PS, and JAK1, as previously demonstrated with genetic and molecular approaches (21, 37–39, 62). In NK and T cells, IL-13 induces the activation of STAT6, probably via IL-4Rα, that is also consistent with the models where IL-4Rα is able to associate with the newly discovered IL-13Rα (28–30). However, it would be interesting to determine whether or not these IL-13Rα(s) are differentially distributed between NK and T cells in the future study. A role for IL-2Rγ (or cγ) in IL-13 signaling also has been suggested based upon IL-13-inducible tyrosine phosphorylation of JAK3 (21) and its association with IL-4Rα (63). In this study, we have demonstrated that IL-13 induced the tyrosine phosphorylation of JAK3 in primary human T cells, although less effectively than IL-4 (Fig. 7), suggesting that common γ might be one of the IL-13R components in T cells, probably in NK cells. In contrast, we did not observe a detectable level of JAK1 tyrosine phosphorylation although all proteins are expressed in equivalent amounts (data not shown). These findings are in contrast to a previous report that showed IL-13 induced JAK1 tyrosine phosphorylation, but not JAK3, in human B cells (62). Thus, distinct patterns of IL-13 and IL-4 signaling may be observed within different cell types although they may share only the common signaling chain, IL-4Rα. In support of this hypothesis, we demonstrate that IL-13, as well as IL-4, distinctively induces cell-specific patterns of Cϵ GAS DNA-protein-binding complexes between NK and T cells.

Previous studies have reported the existence of alternatively spliced forms of STAT proteins such as STAT1α and STAT18 (64), STAT3 (65) and STAT5 (66). Since our results derived from anti-STAT6 supershift analysis suggest unique STAT6 DNA-protein complexes, we hypothesize that these distinct complexes may represent either homodimeric and homotetrimeric forms of STAT6 or different spliced isoforms of STAT6 protein(s). This hypothesis is consistent with the previous observation that the multiple of STAT6 mRNA species were found in spleen, thymus, and peripheral blood lymphocytes (36, 67). Thereby, the activation of distinct splice variants of STAT6 also may explain a putative mechanism by which IL-13 and IL-4 can differentially signal in various type cells, such as NK and T cells in our study, with different biologic effects. However, it is unclear why IL-13 and IL-4 induce similar STAT6 DNA-binding complexes in NK cells, but have distinctive effects on IFN-γ production. Nevertheless, our data suggest that other signal transduction pathway(s) induced by IL-13 or IL-4 also might be involved in regulating IFN-γ production in this cell type.

In summary, we have demonstrated that IL-13, like IL-4, can directly and differentially induce STAT6 activation in freshly isolated primary human T and NK cells, providing new evidence that these cytokines may exert cell-dependent effects on primary lymphocyte populations. In addition, we have established that IL-13 has a unique biologic effect on the modulation of IFN-γ secretion, cytolytic potential, and proliferation of primary human NK and T cells. Therefore, IL-13 may play an important role in the regulation of NK and T cell biologic activities.

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References

1. Ortaldo, J. R., and R. B. Herberman. 1984. Heterogeneity of natural killer cells. *Annu. Rev. Immunol. 2:359.*
2. Trinchieri, G. 1989. Biology of natural killer cells. *Adv. Immunol. 47:187.*
3. Paul, E. W., and R. A. Seder. 1994. Lymphocyte responses and cytokines. *Cell 76:241.*
4. Minty, A., P. Chalon, J.-M. Derocq, X. Dumont, J.-C. Gouillemot, M. Kaghad, C. Labit, P. Leplatois, P. Liauzun, B. Miloux, C. Minty, P. Casellas, G. Loison,

J. Lupker, P. Ferrara, and D. Caput. 1993. Interleukin-13 a new human lymphokine regulating inflammatory and immune response. *Nature 362:248.*
5. Zurawski, G., and J. E. de Vries. 1994. Interleukin 13, an interleukin 4-like cytokine that acts on monocytes and B cells, but not on T cells. *Immunol. Today 15:19.*
6. Zurawki, S. M., F. Vega, Jr., B. Huyghe, and G. Zurawski. 1993. Receptors for interleukin-13 and interleukin-4 cytokine are complex and share a novel component that functions in signal transduction. *EMBO J. 12:2663.*
7. Debinski, W., R. Miner, P. Leland, N. I. Obiri, and R. K. Puri. 1996. Receptor for interleukin (IL) 13 does not interact with IL-4 but receptor for IL-4 interacts with IL-13 on human glioma cells. *J. Biol. Chem. 271:22428.*
8. Burd, P. R., W. C. Thompson, E. E. Max, and F. E. Mills. 1995. Activated mast cells produce interleukin 13. *J. Exp. Med. 181:1373.*
9. Punnonen, J., G. Aversa, B. G. Cocks, A. N. J. McKenzie, S. Menon, G. Zurawski, R. de Waal Malefyt, and J. E. de. Vries. 1993. Interleukin 13 induces interleukin 4-independent IgG4 and IgE synthesis and CD23 expression by human B cells. *Proc. Natl. Acad. Sci. USA 90:3730.*
10. Defrance, T., P. Carayon, G. Billian, J-C. Guillmot, A. Minty, D. Caput, and P. Ferrara. 1994. Interleukin 13 is a B cell stimulating factor. *J. Exp. Med. 179: 135.*
11. Cocks, B. G., R. de Waal Malefyt, J-P. Galizzi, J. E. de. Vries, and G. Aversa. 1993. IL-13 induces proliferation and differentiation of human B cells activated by the CD40 ligand. *Int. Immunol. 5:657.*
12. Aversa, G., J. Punnonen, B. G. Cocks, R. de Waal Malefyt, F. Vega, F. V. Sandra, Jr., S. M. Zyrawski, G. Zurawski, and J. E. de. Vries. 1996. An interleukin 4 (IL-4) mutant inhibits both IL-4 or -13-induced human immunoglobulin G4 (IgG4) and IgE synthesis and B cell proliferation: support for a common component shared by IL-4 and IL-13 receptor. *J. Exp. Med. 178:2213.*
13. de Waal Malefyt, R., J. S. Abrams, S. M. Zurawski, J-C. Lecron, S. Mohan-Peterson, B. Sanjanwala, B. Bennett, J. Silver, J. E. d. Vries, and H. Yassel. 1995. Differential regulation of IL-13 and IL-4 production by human $CD8^+$ and $CD4^+$ Th0, Th1 and Th2 T cell clones and EBE-transformed B cells. *Int. Immunol. 7:1405.*
14. Murata, T., P. D. Noguchi, and R. K. Puri. 1996. Receptors for interleukin (IL)-4 do not associate with the common gamma chain, and IL-4 induces the phosphorylation of JAK2 tyrosine kinase in human colon carcinoma cells. *J. Biol. Chem. 270:30829.*
15. Obiri, N. I., W. Debinski, W. J. Leonard, and R. K. Puri. 1995. Receptor for IL-13. *J. Biol. Chem. 270:8797.*
16. Köhler, I., P. Alliger, A. Minty, D. Caput, P. Ferrara, Höll-Neugebauer, B., G. Rank, and E. P. Rieber. 1994. Human interleukin-13 activates the interleukin-4-dependent transcription factor NF-IL-4 sharing a DNA binding motif with an interferon-(-induced nuclear binding factor. *FEBS Lett. 345:187.*
17. Lin, J-X., T-S. Migone, M. Tsang, M. Griedman, J. A. Weatherbee, L. Zhou, A. Yamauchi, E. T. Bloom, J. Mietz, S. John, and W. J. Leonard. 1995. The role of shared receptor motif and common Stat protein in the generation of cytokine pleiotropy and redundancy by IL-2, IL-4, IL-7, IL-13, and IL-15. *Immunity 2:231.*
18. Kishimoto, T., T. Taga, and S. Akira. 1994. Cytokine signal transduction. *Cell 76:253.*
19. Zurawski, S. M., P. Chomara, O. Djosson, C. Bidaud, A. N. J. McKenzie, P. Miossec, J. Banchereau, and G. Zurawski. 1995. The primary binding subunit of the human interleukin-4 receptor is also a component of the interleukin-13 receptor. *J. Biol. Chem. 270:13869.*
20. Smerz-Bertling, C., and A. Duschi. 1995. Both interleukin 4 and interleukin 13 induce tyrosine. *J. Biol. Chem. 270:966.*
21. Malabarba, M. G., H. Rui, H. H. J. Deutsch, J. Chung, F. S. Kalthoff, W. L. Farrar, and R. A. Kirken. 1996. Interleukin-13 is a potent activator of JAK3 and STAT6 in cells expressing interleukin-2 receptor-γ and interleukin-4 receptor-α. *Biochem. J. 319:865.*
22. Malabarba, M. G., R. A. Kirken, H. Rui, K. Koettnitz, M. Kawamura, J. J. O'Shea, F. S. Kalthoff, and W. L. Farrar. 1995. Activation of JAK3, but not JAK1, is critical to interleukin-4 (IL-4) stimulated proliferation and requires a membrane-proximal region of IL-4Rα. *J. Biol. Chem. 270:9630.*
23. Takeda, K., M. Kamanaka, T. Tanaka, T. Kishimoto, and S. Akira. 1996. Impaired IL-13-mediated functions of macrophages on STAT6-deficient mice. *J. Immunol. 157:3220.*
24. Kotowicz, K., R. E. Callard, K. Friedrich, D. J. Matthews, and N. Klein. 1996. Biological activity of IL-4 and IL-13 on human endothelial cells: functional evidence that both cytokines act through the same receptor. *Int. Immunol. 8:1915.*
25. Takeshita, T., H. Asso, K. Ohtani, N. Ishii, S. Kumaki, N. Tanaka, H. Munakata, M. Najamura, and K. Sugamura. 1992. Cloning of the gamma chain of the human IL-2 receptor. *Science 257:379.*
26. Kondo, M., T. Takeshita, N. Ishii, M. Nakamura, S. Watanabe, K-I. Arai, and K. Sugamura. 1993. Sharing of the interleukin-2 (IL-2) receptor gamma chain between receptors for IL-2 and IL-4. *Science 262:1874.*
27. Miyazaki, T., A. Kawahara, H. Fujii, Y. Nakagawa, Y. Minami, Z-J. Liu, I. Oishi, O. Silvennoinen, B. A. Witthuhn, J. N. Ihle, and T. Taniguchi. 1994. Functional activation of Jak1 and Jak3 by selective association with IL-2 receptor subunits. *Science 266:1045.*
28. Hilton, D. J., J-G. Zhang, D. Metcalf, W. S. Alexander, N. A. Nicola, and T. A. Willson. 1996. Cloning and characterization of a binding subunit of the interleukin 13 receptor that is also a component of the interleukin 4 receptor. *Proc. Natl. Acad. Sci. USA 93:497.*
29. Caput, D., P. Laurent, M. Kaghad, J.-M. Lelias, S. Lefort, N. Vita, and P. Ferrara. 1996. Cloning and characterization of a specific interleukin (IL)-13 binding protein structurally related to the IL-5 receptor α chain. *J. Biol. Chem. 271:16921.*
30. Aman, M. J, N. Tayebi, N. I. Obiri, M. S. Modi, and W. J. Leonard. 1996. cDNA cloning and characterization of the human interleukin 13 receptor α chain. *J. Biol. Chem. 271:29265.*
31. Darnell, J. E., I. M. Kerr, and G. R. Stark. 1994. Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. *Science 264:1416.*
32. Ihle, J. N. 1995. Cytokine receptor signaling. *Nature 377:591.*
33. Taniguchi, T. 1995. Cytokine signaling through nonreceptor protein tyrosine kinases. *Science 268:251.*
34. Russell, S. M., J. A. Johnston, M. Noguchi, M. Kawamura, C. M. Bacon, M. Friendmann, M. Berg, D. W. McVicar, B. A. Witthuhn, O. Silvennoinen, A. S. Goldman, F. C. Schmalstieg, J. N. Ihle, J. J. O'Shea, and W. J. Leonard. 1994. Interaction of IL-2Rβ and γc chains with Jak1 and Jak3: implications for XSCID and XCID. *Science 266:1042.*
35. Kotanides, H., and N. C. Reich. 1993. Requirement of tyrosine phosphorylation for rapid activation of a DNA binding factor by IL-4. *Science 262:1265.*
36. Hou, J., U. Schindler, W. J. Henzel, T-C. Ho, M. Brasseur, and S. L. McKnight. 1994. An interleukin-4-induced transcription factor: IL-4 Stat. *Science 265:17014.*
37. Ryan, J. J., L. C. McReynolds, A. Keegan, L-H. Wang, E. Garfein, P. Rothman, K. Nelms, and W. E. Paul. 1996. Growth and gene expression are predominantly controlled by distinct regions of the human IL-4 receptor. *Immunity 4:123.*
38. Wang, H. Y., W. E. Paul, and A. D. Keegan. 1996. IL-4 function can be transferred to the IL-2 receptor by tyrosine containing sequences found in the IL-4 receptor α chain. *Immunity 4:113.*
39. Lai, S. Y., J. Molden, K. D. Liu, J. M. Puck, M. D. White, and M. A. Goldsmith. 1996. Interleukin-4-specific signal transduction events are driven by homotypic interaction of the interleukin-4 receptor α chain. *EMBO J. 15:4506.*
40. Oakes, S. A., F. Candotti, J. A. Johnston, Y-Q. Chen, J. J. Ryan, N. Taylor, X. Liu, L. Hennighausen, L. D. Notarangelo, W. E. Paul, R. M. Blaese, and J. J. O'Shea. 1996. Signaling via IL-2 and IL-4 in JAK3-deficient severe combined immunodeficiency lymphocytes: JAK3-dependent and independent pathways. *Immunity 5:605.*
41. Taketa, K., T. Tanaka, W. Shi, M. Matsumoto, S-I. Kashiwamura, K. Nakanishi, N. Yoshida, T. Kishmoto, and S. Akira. 1996. Essential role of STAT6 on IL-4 signaling. *Nature 380:627.*
42. Shimoda, K., J. v. Deursen, M. Y. Sangster, S. R. Sarawar, R. T. Carson, R. A. Tripp, C. Chu, F. W. Quelle, T. Nosaka, D. A. A. Vignali, P. C. Doherty, G. Grosveld, W. E. Paul, and J. N. Ihle. 1996. Lack of IL-4-induced Th2 response and IgE class switching in mice with disrupted Stat6 gene. *Nature 380:630.*
43. Kaplan, M. H., U. Schindler, S. T. Smiley, and M. J. Grusby. 1996. STAT6 is required for mediating responses to IL-4 and for the development of Th2 cells. *Immunity 4:313.*
44. Patel, B. K. R., L-M. Wang, C-C. Lee, W. G. Taylor, J. H. Pierce, and W. J. LaRochelle. 1996. Stat6 and Jak1 are common elements in platelet-derived growth factor and interleukin-4 signal transduction pathways in NIH 3T3 fibroblasts. *J. Biol. Chem. 271:22175.*
45. Timomen, T., J. R. Ortaldo, and R. B. Herberman. 1981. Characteristics of human large granular lymphocytes and relationship to natural killer and K cells. *J. Exp. Med. 153:569.*
46. Smyth, M. J., and J. R. Ortaldo. 1991. Comparison of the effect of IL-2 and IL-6 on the lytic activity of purified human peripheral blood large granular lymphocytes. *J. Immunol. 146:1380.*
47. Lozzi, C. B., and B. B. Lozzi. 1975. Human chronic myelogenous leukemia cell line with positive philadelphia. *Blood 45:321.*
48. Mason, L. H., S. K. Anderson, W. M. Yokoyama, H. R. C. Smith, R. Winkler-Pickett, and J. R. Ortaldo. 1996. The Ly-49D receptor activates murine natural killer cells. *J. Exp. Med. 184:2119.*
49. Yu, C-R., J-X. Lin, D. W. Fink, S. Akira, E. T. Bloom, and A. Yamauchi. 1996. Differential utilization of Janus kinase-signal transducer and activator of transcription signal pathways in the stimulation of human natural killer cells by IL-2, IL-12, and IFN-α. *J. Immunol. 157:126.*
50. Rothman, P., S. C. Li, L. Gorham, F. Alt, and M. Boothby. 1991. Identification of a conserved lipopolysaccharide-plus-interleukin-4-responsive element located at the promoter of germ line e transcripts. *Mol. Cell. Biol. 11:5551.*
51. Wagner, B. J., T. E. Hayes, C. J. Hoban, and B. H. Cochran. 1990. The SIE binding element confers sis/PDGF inducibility onto the c-fos promoter. *EMBO J. 9:4477.*
52. Sadowski, H. B., K. Shuai, J. E. Darnell, and M. Z. Giman. 1997. A common nuclear signal transduction pathway activated by growth factor and cytokine receptor. *Science 261:1739.*
53. Nagler, A., L. L. Lanier, and J. H. Phillips. 1988. The effects of IL-4 on human natural killer cells, a potent regulator of IL-2 activation and proliferation. *J. Immunol. 141:2349.*
54. Kawakami, Y., M. C. Custer, S. A. Rosenberg, and M. T. Lotze. 1989. IL-4 regulates IL-2 induction of lymphokine-activated killer activity from human lymphocytes. *J. Immunol. 142:3452.*
55. Vercelli, D., H. H. Jabara, R. P. Lauener, and R. S. Geha. 1990. IL-4 inhibits the synthesis of IFN-γ and induces the synthesis of IgE in human mixed lymphocyte culture. *J. Immunol. 144:570.*
56. Brown, M., J. Hu-Li, and W. E. Paul. 1988. IL-4/B cell stimulatory factor 1 stimulates T cell growth by an IL-2-independent mechanism. *J. Immunol. 131: 504.*

57. Martinez, O. M., R. S. Gibbons, M. R. Garvin, and F. R. Aronson. 1990. IL-4 inhibits IL-2 receptor expression and IL-2-dependent proliferation of human T cells. *J. Immunol. 144:2211.*
58. Ihle, J. N. 1996. STATs: signal transducers and activators of transcription. *Cell 84:331.*
59. Zhong, Z., Z. Wen, and J. E. Darnell. 1994. Stat3: STAT family member activated by tyrosine phosphorylation in response to epidermal growth factor and interleukin-6. *Science 264:95.*
60. Zhang, X., J. Blenis, H-C. Li, C. Schindler, S. Chen-Kiang. 1995. Requirement of serine phosphorylation for formation of STAT-promoter complexes. *Science 267:1990.*
61. Nakayuki, T., J. Blenis, H-C. Li, C. Schlinder, S. Chen-Kiang. 1995. Requirement of serine phosphorylation for formation of STAT-promoter complexes. *Science 267:1990.*
62. Keegan, A. D., J. A. Johnston, P. J. Tortolani, L. J. McReynolds, C. Kinzer, J. J. O'Shea, and W. E. Paul. 1995. Similarities and differences in signaling transduction by interleukin-4 and interleukin-13: analysis of Janus kinase activation. *Proc. Natl. Acad. Sci. USA 92:7681.*
63. Rolling, C., D. Treton, S. Pellegrini, P. Galanaud, and Y. Richard. 1996. IL-4 and IL-13 receptors share the gamma chain and activate STAT6, STAT3 and STAT5 proteins in normal human B cells. *FEBS Lett. 393:53.*
64. Fu, X.-Y., C. Schindler, T. Improta, R. Aebersold, and J. E. Darnell. 1992. The proteins of ISGF-3, the interferon α-induced transcriptional activator, define a gene family involved in signal transduction. *Proc. Natl. Acad. Sci. USA 89:7840.*
65. Schaefer, T. S., L. K. Sanders, and D. Nathans. 1995. Cooperative transcriptional activity of Jun and Stat3β, a short form of STAT3. *Proc. Natl. Acad. Sci. USA 92:9097.*
66. Wang, D., D. Stravopodis, S. Teglund, J. Kitazawa, and J. N. Ihle. 1996. Naturally occurring dominant negative variants of Stat5. *Mol. Cell. Biol. 16: 6141.*
67. Jaso-Friedmann, L., J. H. Leary, III, and D. L. Evans. 1994. Pathways of signaling in nonspecific cytotoxic cells: effects of protein kinase and phosphatase inhibitors and evidence for membrane tyrosine phosphorylation. *Cell. Immunol. 153:142.*