**Brief Definitive Report**

**Stimulation of FcγRIIIA Results in Phospholipase C-γ1 Tyrosine Phosphorylation and p56lc Activation**

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**Summary**

Binding of ligand to the α subunit of FcγRIIIA(CD16), expressed at the natural killer (NK) cell membrane in association with homo or heterodimers of proteins of the γ family, results in phosphorylation of several proteins on tyrosine residues. We have analyzed the role of protein tyrosine phosphorylation in the regulation of molecular events induced upon stimulation of FcγRIIIA in NK cells and in T cells expressing the FcγRIIIα chain in association with endogenous γ2 homodimers and devoid of other (CD3, CD2) transducing molecules. Our data indicate that treatment of these cells with protein tyrosine kinase inhibitors prevents not only FcγRIIIA-induced protein tyrosine phosphorylation but also phosphatidylinositol 4,5 diphosphate hydrolysis and increased intracellular Ca2+ concentration, indicating a primary role of tyrosine kinase(s) in the induction of these early activation events. Occupancy of FcγRIIIA by ligand results in phospholipase C (PLC)-γ1 tyrosine phosphorylation in NK cells and in FcγRIIIA-transfected CD3−/CD2− T cells, and induces functional activation of p56lc in FcγRIIIAα/γ2-transfected T cells, suggesting the possibility that the receptor-induced PLC-γ1 activation occurs upon phosphorylation of its tyrosine residues mediated by this kinase and is, at least in part, responsible for the signal transduction mediated via CD16 upon ligand binding.

The low affinity receptor for the Fc fragment of IgG, FcγRIIIA(CD16), is an oligomeric complex composed of one Fc binding (α) chain associated with homo or heterodimers of the γ and/or γ chain, originally identified as components of the multimeric high affinity IgE receptor (FcεRI) and of the TCR/CD3 complex, respectively. These chains have a primary role to prevent degradation of FcγRIIIA α in the endoplasmic reticulum, thus allowing its expression at the cell membrane, and are responsible for the receptor-mediated signal transduction (for review see reference 1). Most FcγRIIIA on NK cells are associated with γ2, and only a minority of them with γ1 homodimers (2).

FcγRIIIAα/γ2 complexes transduce both early and late activation events similar to those observed in NK cells (3). In these cells, FcγRIIIA stimulation with immune complexes or specific antibodies results in protein tyrosine phosphorylation (4–6), phosphatidylinositol 4,5 diphosphate (PIP2) hydrolysis and increased intracellular Ca2+ concentration ([Ca2+]i) (7) that depends upon both release of Ca2+ from the intracellular stores and extracellular Ca2+ internalization, necessary for transcriptional activation of cytokine genes (7, 8). PIP2 hydrolysis, primarily a consequence of activation of specific phospholipase(s), results in IP3 release and consequent [Ca2+]i increase observed upon receptor occupancy. Protein tyrosine phosphorylation is an early event in FcγRIIIA-dependent signal transduction, and its inhibition abolishes the FcγRIIIA-induced rise in [Ca2+]i and more distal effects in NK cells (6).

In this study, we analyzed the early activation events mediated by FcγRIIIA in NK cells and in CD3−/CD2− cells expressing this receptor in association with γ2 homodimers. Our data using FcγRIIIAα/γ2-transfected CD3−/CD2−/γ− Jurkat T cells indicate that FcγRIIIAα/γ2 complexes transduce signals resulting in the same early biochemical events transduced by FcγRIIIA/γ2 or observed in NK cells upon FcγRIIIA stimulation, and that FcγRIIIA stimulation induces tyrosine phosphorylation of phospholipase C (PLC-γ1) in both cell types and increased p56lc activity in FcγRIIIAα/γ2-expressing cells, suggesting a causal relationship between these events and the production of second messenger molecules.

**Materials and Methods**

**FcγRIII Expression Vectors and DNA Transfection.** The human T cell leukemia-derived Jurkat cell line clone J32.10 and its CD3−/CD2− mutant J32−65.3.1 have been described (9). They were maintained in culture in RPMI-1640 medium (Flow Laboratories Inc., Malvern, VA) supplemented with 10% fetal bovine serum
using a stepwise gradient of increasing concentrations of ammo-
ion-exchange resin (Bio-Rad Laboratories, Richmond, VA) columns
with TCA (10% final concentration) after 45 s incubation.

mycin A were incubated for 3 rain with or without the indicated

Hydrosoluble lipids were extracted and fractionated on AG1-X8
#g/ml) was then added to all samples. Stimulation was stopped

3H-myoinositol (sp act 18.7 Ci/mMol; Amersham Corp.,

Arlington Heights, IL) in the presence or absence of 5 #M her-

bility of time using an LS50 spectrofluorometer and FLDM soft-

OR) as described (7).

[Ca2+]. Measurements. Anti-CD3 (control) and anti-CD16
mAb (5 #g/ml); GaMig (50 #g/ml), and ionomycin (0.5 #g/ml)
were added, as indicated, in the presence or absence of 1 mM ETGA,
to cells loaded with Fura-2/AM (Molecular Probes, Inc., Eugene,
OR) as described (7). [Ca2+]i variations were calculated (7) as a
function of time using an LS50 spectrofluorometer and FLDM soft-
ware (Perkin-Elmer Cetus Corp., Norwalk, CT). Experiments were
also performed on cells cultured (5 x 106/ml, 16 h) with or
without 5 #M herbimycin A (Gibco BRL Life Technology Inc.,
Gaithersburg, MD) or equal concentrations of diluent (DMSO)
also performed on cells cultured (5 x 106/ml, 16 h) with or

mAb, Polyclonal Sens, and Reagents. The mAb used have been
previously characterized: anti-CD16: 3G8, B73.1; anti-CD3: OKT3;
and anti-CD56: B159.5 (12). IgG were prepared using protein
A-Sepharose (Pharmacia Fine Chemicals). The human Ig-adsorbed,
mouse Ig-affinity purified GaMig was produced in our laboratory.

NK Cell Preparations. Buffy coats from healthy donors were
obtained from the American Red Cross (Philadelphia, PA). PBMC
were collected at the interface of Ficoll-Hypaque density gradient
(Lymphoprep, Nyonmed Pharma AS, Oslo, Norway). PBL were
obtained after adherence to plastics. Homogeneous NK cell prep-
arrations were prepared from 30-d cocultures of PBL with RPMI-
8866 B lymphoblastoid cells as described (12).

FcyRIIIA* clones were selected with anti-CD16 mAb and goat
anti-mouse Ig (GaMig)-coated magnetic beads (Dynal Inc., Great
Neck, NY), and cloned by limiting dilution. FcyRIII* clones,
identified by indirect antiglobulin rosetting of anti-CD16 antibody-
sensitized cells (12), were maintained in culture in RPMI-1640 sup-
plemented with 10% protein G (Pharmacia Fine Chemicals, Upps-
sala, Sweden)-adsorbed FBS and 0.4 mg/ml GaG18.

Results and Discussion

FcyRIIIA Expression in Transfected Jurkat T Cells. Flow cy-
ometry analysis of the FcyRIIIA-transfected J32–65.3.1
CD3-/CD2- Jurkat T cells indicated that the receptors ex-
pressed on the cells were resistant to PI-PLC (not shown).
Expression of the transfected FcyRIIIA* cells in these CD3-
cells in the absence of other cotransfected chains occurred
in association with endogenous * chain, as detected in the
anti-CD16 immunoprecipitates from digitonin lysates of the cells
by Western blot analysis with anti-* antiserum (data
not shown), thus reproducing the natural situation of a minor
proportion of FcyRIIIA in NK cells. mRNA transcripts
and protein were not detected in these cells by Northern blot
analysis (data not shown), thus excluding the possibility that
* or */* dimers were associated with FcyRIIIA*.

Induction of Protein Tyrosine Phosphorylation upon FcyRIIIA/
* Stimulation. Western blot analysis with antiphospho-
rosine antiserum was performed on postnuclear supernatants
from lysates of FcyRIIIA* transfected J32–65.3.1 cells after
treatment with anti-CD16 or with control mAb. Upon stim-
ulation of the receptor, several proteins were specifically

nium formate as described (7). [3H]-IP3 and -IP4 (Amersham
Corp.) were run in parallel on separate columns as standards.

Immunoprecipitation and Western Blotting. When indicated, cells
were metabolically labeled with 32P-H3PO4 (sp act 285 Ci/mg;
ICN Biomedicals, Inc.). Briefly, cells were prewashed with
phosphate-free RPMI-1640 medium (ICN Biomedicals, Inc.)
supplemented with 2% diazoy FBS, and incubated (107/ml, 3 h,
37°C in the same medium with 200 #Ci/ml [32P]H3PO4. Labeled
and unlabeled cells (107/ml RPMI-1640) were treated (10 min on
ice) with or without the indicated mAb (ascites, 10-3 dilution),
were lysed in ice-cold medium containing 10 #g/ml GaMig, and
incubated for the indicated times at 37°C. Cells were
lysed with 1% NP-40 in 10 mM Heps, 0.15 M NaCl containing
10% glycerol, 2.5 #g/ml aprotinin, 10 #g/ml leupeptin, 1 mM
PMSF, 1 mM NaVO4, and 5 mM EDTA. Postnuclear supernatants
were precleared (30 min, 4°C) with nonimmune rabbit serum pread-
sorbed on protein A-Sepharose beads (Pharmacia Fine Chemicals)
and precipitated (2 h, 4°C) with the indicated rabbit antiserum, or
normal rabbit serum as control, preadsorbed on protein A-Sepharose
(Pharmacia Fine Chemicals). The precipitated proteins were washed
with lysis buffer, eluted from the beads, and electrophoresed in SDS-
11% or -7.5% polyacrylamide gels under reducing conditions.
Western blot analysis was performed with the indicated antibodies
after transfer of the proteins to nitrocellulose filters (Schleicher
and Schuell, Inc.; Keene, NH). Either 125I-protein A (0.5 #Ci/ml, sp
act 30 #Ci/ #g; ICN Biomedicals, Inc.) or horseradish peroxidase
(HRP)-labeled donkey anti-rabbit serum (Amersham Corp.) were
used for detection by autoradiography or enhanced chemilumines-
cence (ECL; Amersham Corp.), respectively.

In Vitro Kinase Assay. Cells were stimulated and lysed, and
p56* was immunoprecipitated as described above. The immuno-
precipitates were washed five times in 10 mM Heps, 0.15 M NaCl
containing 0.2% NP-40, 10% glycerol, 400 #g NaVO4, 5 #g
MnCl2, and 10 mM MgCl2. The kinase reaction (13) was
performed (15 min, 20°C) in 30 #l of the same buffer in the presence
of 1 #M ATP and 10 #Ci #P[ATP (sp act 4,500 Ci/mMol;
ICN Biomedicals, Inc.). The proteins were then eluted (1% SDS,
30 min, 65°C), electrophoresed in SDS-PAGE, and detected as above.

[Ca2+]i Measurements. Anti-CD3 (control) and anti-CD16
mAb (5 #g/ml); GaMig (50 #g/ml), and ionomycin (0.5 #g/ml)
were added, as indicated, in the presence or absence of 1 mM ETGA,
to cells loaded with Fura-2/AM (Molecular Probes, Inc., Eugene,
OR) as described (7). [Ca2+]i variations were calculated (7) as a
function of time using an LS50 spectrofluorometer and FLDM soft-
ware (Perkin-Elmer Cetus Corp., Norwalk, CT). Experiments were
also performed on cells cultured (5 x 106/ml, 16 h) with or
without 5 #M herbimycin A (Gibco BRL Life Technology Inc.,
Gaithersburg, MD) or equal concentrations of diluent (DMSO)
control. After culture, the cells were >90% viable and surface levels
of FcyRIII and other markers were identical to those on control
nontreated cells, as detected by immunofluorescence.

Phosphatidylinositol Hydrolysis. Duplicate (107) samples of
samples of cells metabolically labeled (16 h, 37°C) with 10 #Ci/ml
3H-myoinositol (sp act 18.7 Ci/mMol; Amersham Corp.,
Arlington Heights, IL) in the presence or absence of 5 #M her-
binycin A were incubated for 3 min with or without the indicated
antibodies (ascites, 10-3 dilution in HBSS supplemented with 1
mM CaCl2, 1 g/liter glucose, and 10 mM LiCl). GaMig (50
#g/ml) was then added to all samples. Stimulation was stopped
with TCA (10% final concentration) after 45 s incubation.
Hydrosoluble lipids were extracted and fractionated on AG1-X8
ion-exchange resin (Bio-Rad Laboratories, Richmond, VA) columns
using a stepwise gradient of increasing concentrations of ammo-
phosphorylated on tyrosine residues (Fig. 1A), one of which was identified as the γ chain by immunoprecipitation (not shown). These data indicate that: (a) FcγRIIIA(CD16) stimulation induces tyrosine phosphorylation independently from coexpression of CD2 and/or CD3; (b) the complex FcγRIIIAα/γ transduces signals similar to those transduced by FcγRIIIAα in association with γ chains; and (c) the signal transduction events mediated by the γ chain in the presence of the cytoplasmic domain of the FcγRIIIA α chain (our model) are qualitatively similar to those reported to be transduced by chimeric FcγRIIIA/γ molecules, like what is observed in models that utilize γ chain (3, 14).

Role of Tyrosine Phosphorylation in FcγRIIIA/γ2-induced PIP2 Hydrolysis and Ca2+ Mobilization. We addressed the question of whether tyrosine kinase activation and PIP2 hydrolysis with consequent Ca2+ mobilization are related or independent events. Stimulation of FcγRIIIAα/γ2 in J32-65.3.1 cells with anti-CD16 mAb followed by crosslinking with GaM Ig induced 326 ± 75% and 171 ± 54% increase (mean ± SD, six experiments) in the production of IP3 and IP4, respectively, compared with that observed in control cells treated with GaM Ig alone. Treatment with control anti-CD56 or anti-CD3 mAb and GaM Ig did not result in increased production of IP3 (109 ± 23%) or IP4 (92 ± 7%). Treatment of the cells with herbimycin A (Fig. 1A) or genistein (data not shown) induced inhibition of constitutive and FcγRIIIA-induced protein tyrosine phosphorylation in a dose-dependent manner. Using 5 μM herbimycin A, no significant increase in IP3 or IP4 over control was observed upon FcγRIIIA stimulation (Fig. 1B).

A prompt increase in [Ca2+]i occurred in the cells treated with anti-CD16 mAb 3G8. This increased further upon cross-linking the Ab with GaM Ig and, like in NK cells (7), was due to both Ca2+ mobilization from intracellular stores and to extracellular Ca2+ internalization, as detected in experiments performed in the presence of EGTA (Fig. 2A). Pretreatment with herbimycin A (Fig. 2B), resulted in a >90% reduction of the [Ca2+]i increase induced upon FcγRIIIA stimulation, as compared with control nontreated cells. Ionomycin induced similar levels of [Ca2+]i increase in both untreated and herbimycin A-treated cells. Identical results were obtained with NK cells (Fig. 2 B). These data indicate that tyrosine phosphorylation is a prerequisite for FcγRIII-mediated signal transduction and suggest that PLC activation upon FcγRIIIA occupancy depends on its induced phosphorylation on tyrosine residues.

Tyrosine Phosphorylation of PLC-γ1 upon FcγRIIIA Stimulation. PLC-γ1 undergoes tyrosine phosphorylation and activation upon stimulation of other receptor systems that either possess intracellular tyrosine kinase domains (for review see reference 15) or associate with and activate tyrosine kinases of the src family (TCR/CD3, FceRI) (16-18). Tyrosine phosphorylation of PLC-γ1 was analyzed in FcγRIIIAα-transfected J32-65.3.1 cells. PLC-γ1 was immunoprecipitated from cells treated or not with anti-CD16 mAb, and GaM Ig and the immunoreactive material was analyzed in Western blotting with antiphosphotyrosine antisera (Fig. 3). A tyrosine-phosphorylated protein with 145–150 kD Mr was detected only in the PLC-γ1 immunoprecipitates from cells treated with anti-CD16 mAb. Using affinity-purified anti-

Figure 1. Effect of tyrosine kinase inhibition of FcγRIIIA-induced protein tyrosine phosphorylation and PIP2 hydrolysis. (A) FcγRIIIAα/γ2-expressing J32-65.3.1 cells were cultured for 16 h without (none) or with the indicated doses of herbimycin A. Cells were treated with anti-CD16 mAb 3G8 and GaM Ig for the indicated times, and lysed. Postnuclear supernatants were electrophoresed in 11% SDS-PAGE. Western blot analysis was performed with antiphosphotyrosine antiserum and 125I-protein A. (B) The cells were labeled with 3H-myoinositol (16-h culture without (medium) or with 5 μM herbimycin A) and then treated with the antibodies indicated at the bottom and GaM Ig. Levels of IP3 (■) and IP4 (□), analyzed by ion exchange chromatography, are reported as the percentage of those in control cells (nontreated cells: IP3, 447 cpm; IP4, 1,002 cpm; herbimycin A-treated cells: IP3, 289 cpm; IP4, 453 cpm).
PLC-γ1 IgG (Fig. 3), a single band was detected, at similar levels in all anti-PLC-γ1 immunoprecipitates, corresponding to a protein with Mₚ identical to that of the phosphoprotein, confirming the identification of the tyrosine phosphorylated protein with PLC-γ1, and excluding that lack of its detection with antiphosphotyrosine Ab in the precipitates from control cells was due to its absence from these precipitates.

Anti-PLC-γ1 immunoprecipitates from lysates of NK cells treated with anti-CD16 mAb and GaMlg (Fig. 3) contained a tyrosine phosphorylated protein with the same mobility of that precipitated from the FcγRIIIA-transfected cells. The same 150-kD protein was detected in antiphosphotyrosine immunoprecipitates from CD16-stimulated FcγRIIIA/γ₂-expressing Jurkat and from NK cells after elution with α-phenylphosphate, SDS-PAGE, and immunoblotting with anti-PLC-γ1 (not shown). However, an additional protein of 140 kD Mₚ was detected in all immunoprecipitates obtained from NK cells, independently from CD16 stimulation, using anti-PLC-γ1 serum, but not using affinity-purified IgG (not shown). The level of reactivity of this protein with the antiphosphotyrosine antibody was significantly increased, compared to controls, in the precipitates from anti-CD16-treated cells, with maximum intensity at 1-min stimulation. Reblotting the same filter with affinity-purified anti-PLC-γ1 IgG we detected a single band (reproducibly in four experiments), that was present with similar intensity in all lanes and corresponded to the 150-kD Mₚ protein.

It is unlikely that the 140-kD species not detected by the affinity-purified anti-PLC-γ1 IgG represents a degradation product with PLC-γ1, and excluding that lack of its detection with antiphosphotyrosine Ab in the precipitates from control cells was due to its absence from these precipitates.

Anti-PLC-γ1 immunoprecipitates from lysates of NK cells treated with anti-CD16 mAb and GaMlg (Fig. 3) contained a tyrosine phosphorylated protein with the same mobility of that precipitated from the FcγRIIIA-transfected cells. The same 150-kD protein was detected in antiphosphotyrosine immunoprecipitates from CD16-stimulated FcγRIIIA/γ₂-expressing Jurkat and from NK cells after elution with α-phenylphosphate, SDS-PAGE, and immunoblotting with anti-PLC-γ1 (not shown). However, an additional protein of 140 kD Mₚ was detected in all immunoprecipitates obtained from NK cells, independently from CD16 stimulation, using anti-PLC-γ1 serum, but not using affinity-purified IgG (not shown). The level of reactivity of this protein with the antiphosphotyrosine antibody was significantly increased, compared to controls, in the precipitates from anti-CD16-treated cells, with maximum intensity at 1-min stimulation. Reblotting the same filter with affinity-purified anti-PLC-γ1 IgG we detected a single band (reproducibly in four experiments), that was present with similar intensity in all lanes and corresponded to the 150-kD Mₚ protein.

It is unlikely that the 140-kD species not detected by the affinity-purified anti-PLC-γ1 IgG represents a degradation product with PLC-γ1, and excluding that lack of its detection with antiphosphotyrosine Ab in the precipitates from control cells was due to its absence from these precipitates.
product of PLC-γ1, specifically occurring in NK cells and detected by the immune serum. Unlike PLC-γ1, this protein appears phosphorylated on tyrosine residues in unstimulated cells. Instead, it is likely to represent a distinct PLC-γ1 homologous phosphoprotein, hyperphosphorylated upon FcγRIIIA stimulation, that crossreacts with the non-affinity purified serum and that is absent in Jurkat T cells, excluding nonspecific reactivity of the serum with a ubiquitous protein. The biochemical characteristics of the 140-kD phosphoprotein suggest its possible relation with PLC-γ2, reported as the major PLC species involved in PIP2 hydrolysis mediated via Slg stimulation in murine B cells (19). The nature of this phosphoprotein, likely to be involved in the FcγRIIIA-mediated signal transduction in NK cells, is under investigation.

In several receptor systems PIP2 hydrolysis, IP3 production, and consequent release of Ca2+ from the intracellular stores observed upon receptor occupancy originate from the activation of PLC-γ1, which depends on its phosphorylation on tyrosine residues occurring shortly after ligand–receptor interaction (17, 20). Our data demonstrate that, like in those systems, FcγRIIIA stimulation induces phosphorylation of PLC-γ1 on tyrosine residues both in NK and in FcγRIIIAα-transfected Jurkat cells, strongly suggesting that induced PLC-γ1 activation may be responsible for the FcγRIIIA-induced PIP2 hydrolysis observed in these cells.

p56κκ Activation upon Occupancy of FcγRIIIA/γc. The kinase(s) responsible for the protein tyrosine phosphorylation induced upon FcγRIIIA stimulation in NK cells has not been identified yet. p56κκ is expressed, with other kinases of the src family, in both NK and T cells (13, and our unpublished data) and has been reported associated with CD4, CD8, and IL-2R p70 chain (21, 22). Its increased activity upon stimulation of these receptors results in auto- and substrate tyrosine phosphorylation. We investigated the participation of p56κκ in the FcγRIIIA/γc-mediated signal transduction in intact cells. p56κκ was immunoprecipitated from FcγRIIIAα-transfected J32–65.3.1 cells metabolically labeled with 32P-orthophosphate and treated with anti-CD16 or control anti-CD56 mAb and GaMlg. The results of one experiment representative of three performed is shown in Fig. 4 B. Kinase activity and p56κκ phosphorylation were evident in all precipitates but were greater in those from cells treated for 5 and 15 min with anti-CD16 mAb.

The data reported here indicate that phosphorylation of p56κκ occurs both in intact cells upon FcγRIIIA stimulation and in vitro, supporting a functional association of this kinase with FcγRIIIA, and suggesting the possibility that p56κκ is at least partially responsible for the observed protein tyrosine phosphorylation upon stimulation of the receptor. Although this may suggest a causal relationship between p56κκ and PLC-γ1 activation, the kinetics of induction of p56κκ activity upon FcγRIIIA stimulation observed in vitro kinase assay is slower than that of FcγRIIIA-induced phosphorylation of most proteins and does not seem compatible with a direct effect of this kinase. However, we cannot exclude that activation of kinases other than p56κκ, as well as the activity of cellular phosphatases, may modify, in the intact cells, the kinetics of protein tyrosine phosphorylation. The experiments performed using metabolically labeled cells indicate that p56κκ is involved in FcγRIIIA signal transduction, but do not allow us to discriminate which amino acid residues are phosphorylated upon receptor stimulation in the intact cells. In these, phosphorylation on serine and/or threonine residues, or differential phosphorylation of negative regulatory tyrosine residues (23) could occur, and possibly modulate the kinetics of enzyme activity. Our data suggest the possibility that p56κκ is physically associated, or induced to be associated upon ligand binding, with FcγRIIIAαc, either directly, or indirectly via γ and/or γ chains or other molecules. They do not exclude, however, that receptor-mediated p56κκ activation is induced indirectly via activation and/or association of this to another kinase(s). Further analysis is needed to determine whether additional or different molecules operate and/or physically associate with the different FcγRIIIA types in NK cells and macrophages.

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References

1. Ravetch, J.V., and J.-P. Kinet. 1991. Fc Receptors. Annu. Rev. Immunol. 9:457.
2. Leturneur, O., I.C.S. Kennedy, A.T. Brini, J.R. Ortiz, J.J. O'Shea, and J.-P. Kinet. 1991. Characterization of the family of dimers associated with Fc receptors (FcεRI and FcγRIII). J. Immunol. 147:2652.
3. Wirthmueller, U., T. Kurosaki, M.S. Murakami, and J.V. Ravetch. 1992. Signal transduction by FcγRIII (CD16): a complex in human natural killer cells. Induction by antibody-dependent cytotoxicity but not natural killing. J. Immunol. 146:206.
4. O'Shea, J.J., A.M. Weissman, I.C.S. Kennedy, and J.R. Ortaldo. 1991. Engagement of the natural killer cell IgG Fc receptor results in tyrosine phosphorylation of the γ chain. J. Exp. Med. 175:1381.
5. O'Shea, J.J., D.W. McVicar, D.B. Kuhns, and J.R. Ortaldo. 1991. Tyrosine phosphorylation of the FcγRIII(CD16): γ complex in human natural killer cells. Induction by antibody-dependent cytotoxicity but not natural killing. J. Immunol. 146:206.
6. Vivier, E., P. Morin, C. O'Brient, B. Drucker, S.F. Schlossman, and P. Anderson. 1991. Tyrosine phosphorylation of the FcγRIII(CD16): γ complex in human natural killer cells. Induction by antibody-dependent cytotoxicity but not natural killing. J. Immunol. 146:206.
7. Cassatella, M.A., I. Anegón, M.C. Cuturi, P. Griskey, G. Trinchieri, and B. Perussia. 1988. FcγR (CD16) interaction with ligand induces Ca2+ mobilization and phosphoinositide turnover in human natural killer cells. Role of Ca2+ in FcγR-(CD16)-induced transcription and expression of lymphokine genes. J. Exp. Med. 167:549.
8. Anegón, I., M.C. Cuturi, G. Trinchieri, and B. Perussia. 1988. Interaction of Fc receptors (CD16) with ligands induces transcription of IL-2 receptor (CD25) and lymphokine genes and expression of their products in human natural killer cells. J. Exp. Med. 167:452.
9. Makni, H., J.S. Malter, J.C. Reed, S. Nobushiko, G. Lang, D. Kioussis, G. Trinchieri, and M. Kamoun. 1991. Reconstitution of an active surface CD2 by DNA transfer in CD2−CD3+ Jurkat cells facilitates CD3-T cell receptor-mediated IL-2 production. J. Immunol. 146:2522.
10. Ravetch, J.V., and B. Perussia. 1989. Alternative membrane forms of FcγRIII (CD16) on human natural killer cells and neutrophils. Cell-type specific expression of two genes that differ in single nucleotide substitutions. J. Exp. Med. 170:481.
11. Greaves, D.R., F.D. Wilson, G. Lang, and D. Kioussis. 1989. Human CD2 3'-flanking sequences confer high-level T cell specific, position-independent gene expression in transgenic mice. Cell. 56:779.
12. Perussia, B., C. Ramon, I. Anegón, M.C. Cuturi, J. Faust, and G. Trinchieri. 1987. Preferential proliferation of natural killer cells among peripheral blood mononuclear cells cocultured with B lymphoblastoid cell lines. Nat. Immun. Cell Growth Regul. 6:171.
13. Veillette, A., M.A. Bookman, E.M. Horak, and J.B. Bolen. 1988. The CD4 and CD8 T cell surface antigens are associated with the internal membrane tyrosine-protein kinase p56lck. Cell. 55:301.
14. Romeo, C., M. Amitiot, and B. Seed. 1992. Sequence requirements for induction of cytolyis by the T cell receptor antigen/Fc receptor γ chain. Cell. 68:889.
15. Weis, A., G. Koretsky, R. Schatzman, and M. Kadlec. 1991. Functional activation of the T cell antigen receptor induces tyrosine phosphorylation of phospholipase C-γ1. Proc. Natl. Acad. Sci. USA. 88:5484.
16. Mustelin, T., K.M. Koggeshall, N. Isakov, and A. Altman. 1990. T cell antigen receptor mediated activation of phospholipase C requires tyrosine phosphorylation. Science (Wash. DC). 247:1584.
17. Park, D.J., H.K. Min, and S.G. Rhee. 1991. IgE-induced tyrosine phosphorylation of phospholipase C-γ1 in rat basophilic leukemia cells. J. Biol. Chem. 266:24237.
18. Hemple, W.H., R.C. Schatzman, and A.L. DeFranco. 1992. Tyrosine phosphorylation of phospholipase C-γ2 upon cross-linking of membrane Ig on murine B lymphocytes. J. Immunol. 148:3021.
19. Kim, H.K., J.W. Kim, A. Zilberstein, B. Margolis, J.G. Kim, J. Schlessinger, and S.G. Rhee. 1991. PDGF stimulation of inositol phospholipid-specific phospholipase C-γ2 in rat basophilic leukemia cells. J. Biol. Chem. 266:24237.
20. Barber, E.K., J.D. Dasgupta, S.F. Schlossman, J.M. Trevillyan, and C.E. Rudd. 1989. The CD4 and CD8 antigens are coupled to a protein-tyrosine kinase (p56lck) that phosphorylates the CD3 complex. Proc. Natl. Acad. Sci. USA. 86:3277.
21. Hatakeyama, M., T. Kono, N. Kobayashi, A. Kawahara, S.D. Levin, R.M. Perlmutter, and T. Taniguchi. 1991. Interaction of the IL-2 receptor with the src-family kinase p56lck. Immunol. Rev. 123:1523.
22. Hurley, T.R., and B.M. Sefton. 1989. Analysis of the activity and phosphorylation of the tk protein in lymphoid cells. Oncogene. 4:265.