A human immunoglobulin G receptor exists in both polypeptide-anchored and phosphatidylinositol-glycan-anchored forms

(cDNA/CD16/phospholipase C/neutrophils/natural killer cells)

BERNARD J. SCALLON*, EILEEN SCIGLIANO†, VICTORIA H. FREEDMAN†, MAY C. MIEDEL‡, YU-CHING E. PAN‡, JAY C. UNKELESS†, AND JAREMA P. KOCHAN*§

Departments of *Molecular Genetics and ‡Protein Biochemistry, Hoffmann–La Roche Inc., Nutley, NJ 07110; and †Department of Biochemistry, Mount Sinai Medical Center, New York, NY 10029

ABSTRACT Several cDNA clones encoding the human immunoglobulin G receptor CD16 were isolated from human lung or peripheral blood leukocyte cDNA libraries. Nucleotide sequence comparisons revealed that the cDNAs could be divided into two groups. cDNA clones in one group encode a protein that terminates 4 amino acids after the putative transmembrane domain. Clones in the second group encode a protein with an extra 21 amino acids that could comprise a cytoplasmic domain. Direct peptide sequencing was used to determine the N terminus of the mature CD16 receptor protein and supported the existence of the two forms of the receptor. Treatment of neutrophils with phosphatidylinositol-specific phospholipase C resulted in the release of a large percentage of the CD16 molecules from the cell surface. In contrast, treatment of natural killer cells with phosphatidylinositol-specific phospholipase C did not release any CD16 from the cell surface. These data demonstrate that both polypeptide-anchored and phosphatidylinositol-glycan-anchored forms of the CD16 molecule exist and that they are differentially expressed on neutrophils and natural killer cells.

One of two known low-affinity receptors for the Fc portion of human immunoglobulin G (IgG) is CD16, otherwise known as FcγRIII. It is a 50- to 70-kDa glycoprotein present on natural killer (NK) cells, neutrophils, and tissue macrophages (1, 2). Studies using monoclonal antibodies (mAbs) against CD16 have established this receptor's role in removing immune complexes from circulation (3) and in mediating antibody-dependent cellular cytotoxicity (2, 4, 5). Although little is known about how IgG binding to CD16 triggers cytolytic functions, cross-linking the receptor on NK cells leads to rapid release of intracellular Ca^{2+} stores (6).

Recent studies have indicated that CD16 is polymorphic among individuals (7) and that NK cells and granulocytes contain forms of the receptor that are both structurally and functionally distinct (8). Treatment of the CD16 antigen on NK cells with N-Glycanase to remove carbohydrate moieties resulted in the production of proteins with apparent molecular masses of 36, 40, and 44 kDa, whereas the same treatment of the granulocyte antigen resulted in the production of 28- and 29-kDa proteins. These differences in CD16 structure probably correlate with functional differences. For example, NK cells lysed anti-CD16 hybridoma cell lines, whereas CD16⁺ granulocytes did not (8).

Several groups have demonstrated that CD16 is anchored to the plasma membrane by a phosphatidylinositol-glycan (PI-G) linkage. Anti-CD16 mAbs have been used to show that a large proportion of immunoprecipitated cell surface antigen on neutrophils is sensitive to phosphatidylinositol-specific

phospholipase C (PI-PLC) (9, 10). In addition, a cDNA clone encoding CD16 directs the expression of a PI-PLC-sensitive receptor in transfected COS cells (11). However, it has not been determined whether polypeptide-anchored forms of CD16 exist. We report here the characterization of cDNA clones that encode polypeptide-anchored forms of CD16¶ and show that CD16 on NK cells is resistant to PI-PLC, whereas a large percentage of the CD16 molecules on neutrophils is indeed susceptible to PI-PLC cleavage.

MATERIALS AND METHODS

Reagents. A human lung cDNA library prepared in λgt11 was purchased from Clontech. A human peripheral blood leukocyte (PBL) cDNA library in λgt11 was provided by Ueli Gubler (Hoffmann–La Roche). Phycoerythrin (PE)-conjugated anti-Leu-19 and anti-CD16 mAbs anti-Leu-11a (a mouse IgG1) and anti-Leu-11b (a mouse IgM) were from Becton Dickinson. mAb 3G8 (a mouse IgG1) has been described (1). Alkaline phosphatase-conjugated goat anti-mouse second antibodies were obtained from Jackson ImmunoResearch. SP6 and T7 promoter primers, pGem-4Z plasmid, and the alkaline phosphatase color reagents nitro-blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate were purchased from Promega Biotec. Sequenase DNA sequencing kit was from United States Biochemical.

Isolation and Sequencing of cDNA Clones. An oligonucleotide corresponding to the transmembrane domain of the rat FcεRI, human FcεRI, and mouse FcγRα with the sequence
$$\text{AA} \overset{G}{\underset{T}{C}} \text{CC} \overset{G}{\underset{A}{A}} \text{GT} \overset{A}{\underset{G}{G}} \text{TCCAC} \overset{A}{\underset{T}{C}} \text{GCAAACAG}$$
was synthesized and end-labeled with [γ-³²P]ATP and T4 kinase. A human lung cDNA library in λgt11 was screened by plaque hybridization with either the oligonucleotide or subsequently isolated cDNA inserts as probe. cDNA inserts were subcloned into pGem-4Z and sequenced by the dideoxy chain termination method using Sequenase.

Expression in *Escherichia coli*. Different CD16 cDNA fragments were expressed in *E. coli*, and extracts were prepared as previously described (12). Recombinant proteins were tested by Western blot analysis for reactivity to mAbs anti-Leu-11b and anti-Leu-11a (4 μg/ml) and rabbit polyclonal anti-CD16 sera (1:300 dilution). Immunoreactive bands were detected by using the appropriate alkaline phosphatase-conjugated antibody as previously described (13).

Purification of CD16. Cells from leukophoresis of a patient with accelerated phase chronic myelogenous leukemia were

---

washed with phosphate-buffered saline (PBS) and lysed in PBS containing 1% Nonidet P-40 and 2 mM diisopropyl fluorophosphate (buffer A). The lysate was clarified by centrifugation at 40,000 × g for 1 hr, preadsorbed to Sepharose 4B, and loaded onto a 20-ml column of 3G8 IgG coupled to Sepharose 4B at 2 mg/ml. The column was washed extensively with buffer A containing 0.2% SDS and finally with 100 ml of 10 mM octyl β-D-thiolglucoside in PBS. CD16 was eluted with 1 M acetic acid containing 10 mM octyl β-D-thiolglucoside and neutralized with 2 M Tris.

Peptide Sequencing. Tryptic digestion of immunoaffinity-purified CD16 was performed in 0.2 M NH₄HCO₃ at a substrate-to-enzyme ratio of 30:1 (wt/wt) at 37°C for 20 hr. Sequence analysis was performed by using a model 470A gas phase sequencer from Applied Biosystems (14).

Cell Preparation. Normal human leukocytes were prepared from buffy coats from whole blood obtained from the Mount Sinai School of Medicine Blood Center. Polymorphonuclear leukocytes were isolated on a two-step Ficoll sodium diatrizoate (Histopaque; Sigma) gradient as described (15). Cytospin preparations of Wright–Giemsa stained cells revealed >99% neutrophils. A fraction enriched for NK cells (large granular mononuclear cells) was prepared from buffy coats according to a modification of the method of Timonen and Saksela (16). Cytospin preparations of this enriched fraction showed 40% monocytes, 36% small nature lymphocytes, and 24% large mononuclear cells with nuclear morphology distinct from that of either monocytes or lymphocytes.

PI-PLC Digestion. Aliquots of cells were incubated with *Bacillus thuringiensis* PI-PLC (17), generously provided by M. Low (Columbia University), at 10 μmol/min per ml of enzyme activity, for 1 hr at 37°C. Incubation of cells with a 2-fold higher concentration of PI-PLC did not result in any detectable differences.

Flow Cytofluorometric Analysis. The following antibodies were used: fluorescein isothiocyanate (FITC)-conjugated 3G8 (1), PE-conjugated anti-Leu-19, DHK 109.3 (a murine IgG1 used as an isotype control), and W6/32 (an anti-human class I murine antibody). Cell pellets were washed, incubated with antibody at a concentration of 10 μg/ml (determined to be saturating amounts of antibody) in PBS containing 0.5 mg/ml human serum albumin, 3 mM glucose, and 0.1% sodium azide for 1 hr at 4°C, and then washed and fixed in 1% neutral buffered formalin. When antibody was not directly conjugated with fluorochrome, FITC-conjugated F(ab')₂ goat anti-mouse IgG was employed. Cells were analyzed in a Coulter electronically programmable individual cell sorter cytofluorograph.

RESULTS

cDNA Isolation and Characterization. Previous analyses of DNA clones encoding receptors for mouse IgG (18), human IgE (19, 20), and rat IgE (20, 21) revealed complete conservation of the sequence Leu-Phe-Ala-Val-Asp-Thr-Gly-Leu in the transmembrane domain. With the prediction that a human

FcγR would also contain this sequence, corresponding oligonucleotides were synthesized and used to screen a human lung cDNA library for FcγR clones. Two hybridizing clones, HL1 and HL12 (0.5- and 1.1-kilobase inserts, respectively), were shown by DNA sequence analysis to encode the same transmembrane sequence described above (Figs. 1 and 2). The clones had identical sequences in the region that they overlapped (nucleotides 497–997).

The nucleotide sequence of the HL12 insert predicted a protein with 178 amino acids of an extracytoplasmic domain (at its 5' end) followed by a 21-amino acid transmembrane domain, a 25-amino acid cytoplasmic tail, and 457 nucleotides of 3' untranslated sequence. That HL12 may encode an immunoglobulin receptor was indicated by the characteristic conserved cysteine residues (Fig. 2) and the 30–35% sequence identity that the extracytoplasmic portion of this protein shares with the mouse (18) IgG Fc receptors and human (19, 20) and rat (20, 21) IgE Fc receptors. The cytoplasmic domain of this protein shows 33% and 38% sequence identity to the corresponding domains of the mouse (18) and rat (M. Nettleton and J.P.K., unpublished observations) IgG Fc receptors, respectively.

Identification of cDNAs as CD16 Clones. To determine whether HL12 encoded FcγRIII (CD16), the 538-base-pair EcoRI/Kpn I fragment (encoding amino acids 31–207) was expressed (in correct and inverted orientation) as a fusion protein in E. coli. Western blot analysis of the E. coli extracts using anti-CD16 mAb anti-Leu-11b or anti-Leu-11a showed strong reactivity to the fusion protein encoded by the plasmid with the insert in the correct orientation (Fig. 3, lanes 2 and 6) but very little reactivity to the control fusion protein (Fig. 3, lane 1). These mAbs also reacted strongly with a nonfusion form of the same recombinant protein (Fig. 3, lanes 5 and 9), demonstrating specificity to the cDNA-encoded portion of the protein. No immunoreactivity was detected when the blotted proteins were incubated only with second antibodies (Fig. 3, lanes 10 and 11) or with unrelated mouse mAbs (data not shown).

Two smaller fragments were also expressed to map further the sites reactive with the mAbs. The fusion protein encoded by the EcoRI/Pvu II fragment of HL12 (amino acids 31–129) did react with polyclonal rabbit anti-CD16 sera (data not shown) but showed very little reactivity with the mAbs (Fig. 3, lanes 4 and 8). In contrast, the protein produced from the EcoRI/Kpn I fragment of HL1 (amino acids 134–207) reacted with both mAbs as strongly as the protein containing amino acids 31–207. These results indicated that HL12 encodes part of the CD16 IgG Fc receptor and that the epitopes on the fusion protein recognized by anti-Leu-11b and anti-Leu-11a map to a 74-amino acid stretch adjacent to the transmembrane region.

Sequence Heterogeneity. The existence of variant forms of CD16 has been established by serological and biochemical analyses (7, 8). To investigate this heterogeneity, three other cDNA clones isolated from the human lung library were
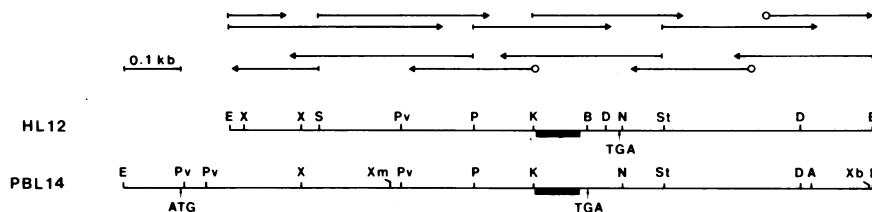

FIG. 1. Restriction maps and sequencing strategy of cDNA clones encoding the conserved sequence Leu-Phe-Ala-Val-Asp-Thr-Gly-Leu. The black box represents the hydrophobic (transmembrane) region. The positions of the translation start and stop codons are shown. The EcoRI sites at the left ends are derived from synthetic linkers used during library construction, whereas the EcoRI sites at the right ends are encoded by the mRNAs. Arrows with open circles represent sequences primed with synthetic oligonucleotides; the remaining sequences were primed with SP6 or T7 promoter primers in smaller subclones. A, Ava I; B, BstBI; D, Dra I; E, EcoRI; K, Kpn I; N, Nco I; P, Pst I; Pv, Pvu II; S, Sal I; St, Stu I; X, Xho I; Xb, Xba I; Xm, Xmn I; kb, kilobase.
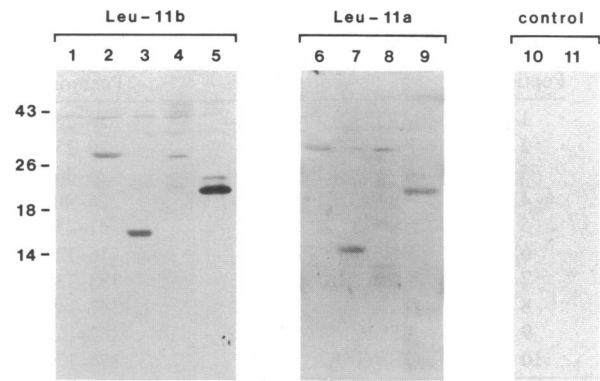
FIG. 3. Western blot analysis. Extracts of *E. coli* expressing different fragments of the cDNA clones were fractionated through a SDS/15% polyacrylamide gel, transferred to nitrocellulose, and reacted with the specified mAb and the appropriate alkaline phosphatase-conjugated second antibody. Different amounts of extract were loaded, such that equivalent amounts of recombinant protein were present in each lane. Leu-11b and Leu-11a, anti-CD16 mAbs; control, conjugated second antibody only. Lanes: 1, EcoRI/Kpn I fragment of HL12 expressed in inverted orientation (12-kDa fusion protein); 2, 6, and 10, EcoRI/Kpn I fragment of HL12 (amino acids 31–207, 29 kDa) in correct orientation; 3, 7, and 11, EcoRI/Kpn I fragment of HL1 (amino acids 134–207, 16 kDa); 4 and 8, EcoRI/Pvu II fragment of HL12 (amino acids 31–129, 23 kDa); 5 and 9, EcoRI/Kpn I fragments of HL12 expressed as nonfusion protein (amino acids 31–207, 23 kDa). Molecular sizes (in kDa) are indicated at left.

FIG. 2. DNA sequence and deduced amino acid sequence of cDNA clones HL12 and PBL14. The cysteine residues typical of FcR genes are boxed, and the transmembrane region in HL12 is underlined.

characterized. DNA sequence analysis revealed that they all contained the same 3′ end as HL12 (delineated by a naturally-occurring EcoRI site) but extended various lengths upstream (starting at nucleotides 445, 744, and 887). The three clones had sequences identical to HL12 except for one clone, which differed by one base at position 1182 in the 3′ untranslated region.

Because sequence heterogeneity was not apparent in the five clones analyzed, the possibility that the clones were unique to that library was considered. To test this hypothesis, a second cDNA library made from mRNA of PBL cells (pooled from many individuals) was screened with the nick-translated 430-base-pair EcoRI/Pst I fragment from the 5′ end of HL12. Three clones, PBL13, PBL14, and PBL17, were isolated and characterized. PBL13 contained a 1.0-kilobase insert identical to nucleotides 289–1326 of HL12. PBL14 and PBL17 appeared identical to each other but different from HL12 as determined by restriction map analysis (Fig. 1). Sequence analysis of the PBL14 insert showed

that it terminated at the same EcoRI site that marked the 3′ end of the other clones but extended 188 nucleotides beyond the 5′ end of HL12. There were a total of 28 base differences between PBL14 and HL12; of the 10 located within the PBL14 coding region, 8 affected an amino acid substitution (of which only 2 would be considered conservative). One resulted in the presence or absence of a potential N-linked carbohydrate site at amino acid position 82. Most strikingly, the difference at nucleotide position 798 resulted in a translation stop signal 21 codons before the translation stop signal in HL12 (Fig. 2). This PBL14-encoded protein, which would lack the cytoplasmic domain, is identical to the predicted sequence reported by Simmons and Seed (11) except at amino acid positions 122 and 151. Interestingly, the 2 amino acids encoded by their clone at these positions are the same as those encoded by HL12. The presence of a cytoplasmic tail in the HL12 gene product, but not in the PBL14 gene product, and the 8-amino acid differences encoded by the two clones confirmed that at least two different forms of CD16 mRNAs exist.

Peptide Sequencing. To identify the N terminus of the mature CD16 molecule and to investigate further the heterogeneity predicted by the cDNA clones, mAb 3G8 was used to immunoaffinity purify CD16 from PBL cells of a patient with chronic myelogenous leukemia. Purified CD16 was digested with trypsin, and several of the resulting peptide fragments were sequenced (Table 1). All peptide sequences corresponded to sequences predicted from PBL14 or HL12, confirming that the cDNAs encode CD16. The sequence of peptide 1 was derived from an incompletely digested fragment and corresponds to the mature N terminus as confirmed by sequencing the intact protein. Thus the methionine residue at position 18 (Fig. 2) was identified as the N-terminal residue of the mature protein. The methionine at position 1 is likely the translation initiation site, indicating that the initial translation product contains a 17-amino acid signal peptide.

Peptide 3 contained an arginine residue at position 36 corresponding to the residue encoded by HL12, whereas peptide 4 contained a serine at this position corresponding to

Table 1. N-terminal sequences of peptides obtained from trypsin-treated purified CD16 protein

Peptide	Sequence	Position*
1	†MRTEDLPK	18–25
2	TEDLPK	20–25
3	AVVFLEPQWYR	26–36
4	AVVFLEPQWYSVLEK	26–40
5	DSVTL	41–45
6	?VFKEEDPI	116–124
7	NTALHK	133–138
8	DRK	147–149
9	ATLK	162–165
10	GLYGSK	174–179

Amino acids with a single underline are encoded by cDNA clone PBL14 but are not encoded by cDNA clone HL12. Amino acids with a double underline are encoded by HL12 but not by PBL14. The sequences of peptides 3 and 10 were each derived from two independent CD16 trypsin digests. Amino acids are identified by the single-letter code.
*See Fig. 2 for location.
†N terminus of mature protein.

the residue encoded by PBL14. These particular peptide sequences were derived from two independent sequence analyses, and there were no ambiguities in identifying the residues as arginine or serine. Peptide 6 contained an aspartic residue at position 122 corresponding to the residue encoded by HL12. Peptides 4, 8, and 10 were identical to the sequence predicted from HL12 except for single amino acid residues (underlined residues), which are predicted from the sequence of PBL14. Peptides 1–4 contain N-terminal sequences not encoded by the partial cDNA insert of HL12.

Analysis of CD16 on Cells. At least a portion of the CD16 population is known to be anchored to the cell surface by a PI-G linkage as shown by sensitivity to PI-PLC digestion (9–11). However, the observation that HL12 predicts a receptor with a cytoplasmic tail prompted us to predict that some CD16 molecules may be anchored by a polypeptide transmembrane domain and therefore be resistant to PI-PLC. We analyzed CD16 on purified neutrophils and NK-enriched cells for that type of anchor. Greater than 99% of human neutrophils bear CD16 as visualized with FITC-conjugated mAb 3G8 (Fig. 4C). Following treatment with PI-PLC, the average amount of CD16 on the surface of the neutrophils, calculated from the mean fluorescence channel, was reproducibly (three experiments) decreased by about 50%. The breadth of the profile after digestion indicated that sensitivity of CD16 on individual cells to PI-PLC was variable. These results were consistent with previously published observations (10). Twenty percent of the neutrophils did not stain after PI-PLC digestion (Fig. 4D). In contrast, PI-PLC had no effect on the staining of neutrophils with the anti-human class I mAb W6/32 (Fig. 4 A and B).

The pattern of staining of NK cells after PI-PLC digestion was markedly different from neutrophils. To facilitate identification of NK cells, two-color fluorescence was performed using FITC-conjugated 3G8 and PE-conjugated anti-Leu-19, which recognizes the NK antigen NHK-1. The enriched NK preparation, gated for granular lymphocytes, gave a population with 66% (Fig. 5C) to 75% (Fig. 5D) double staining cells. The peak staining by both FITC-conjugated 3G8 and PE-conjugated anti-Leu-19 was slightly increased by PI-PLC treatment of the cells and was observed in all three experiments. As before, there was no effect by PI-PLC digestion on the binding of anti-class I mAb (Fig. 5 A and B).

DISCUSSION

A mixture of oligonucleotides corresponding to part of the transmembrane region conserved in the mouse IgG Fc re-
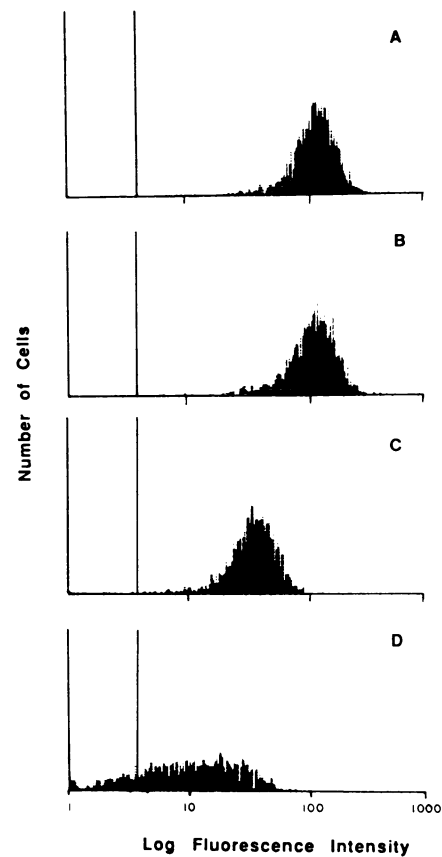

FIG. 4. Expression of polymorphonuclear leukocyte surface antigens before and after PI-PLC digestion. Cells were stained with FITC-conjugated 3G8 (anti-CD16) prior to (C) and following (D) PI-PLC digestion. Control leukocytes were stained with anti-class I antibody W6/32 before (A) and after (B) PI-PLC digestion. Data shown are from one representative experiment.

ceptor (18) and human (19, 20) and rat IgE Fc receptors (20, 21) was used to screen human lung and human PBL cDNA libraries for clones encoding IgG receptors. Positive clones were isolated and identified as CD16 cDNAs as a result of reactivity of their *E. coli* expression products with anti-CD16 mAbs. Their identity was confirmed by comparison of their deduced amino acid sequence with the sequence determined from the native protein. Two clones were very similar to the one reported by Simmons and Seed (11) encoding a PI-G-anchored CD16 molecule. However, we also isolated, from both cDNA libraries, clones encoding CD16 molecules with an alternative C terminus of an additional 21 amino acids. These proteins could presumably be anchored by means of a polypeptide transmembrane domain. Consistent with the existence of polypeptide-anchored forms, CD16 molecules on the surface of NK cells were not sensitive to PI-PLC in our studies. We cannot exclude the possibility that PI-G-anchored CD16 molecules are present on the surface of NK cells and that they are resistant to PI-PLC treatment as has been demonstrated for other proteins (22). CD16 on neutrophils, on the other hand, were sensitive to PI-PLC in agreement with previous reports (9–11) that PI-G-anchored forms exist. The observation that N-Glycanase-treated CD16 molecules isolated from NK cells had an apparent molecular mass greater than similarly treated molecules from granulocytes (8) may be explained by the presence of a polypeptide-anchored form in NK cells and a PI-G-anchored form in granulocytes. Together these results clearly demonstrate the presence of more than one form of CD16 in different cell types. In addition, it is interesting to note that five independent cDNA clones were isolated from a human lung cDNA

Immunology: Scallon *et al.*

Proc. Natl. Acad. Sci. USA 86 (1989) 5083

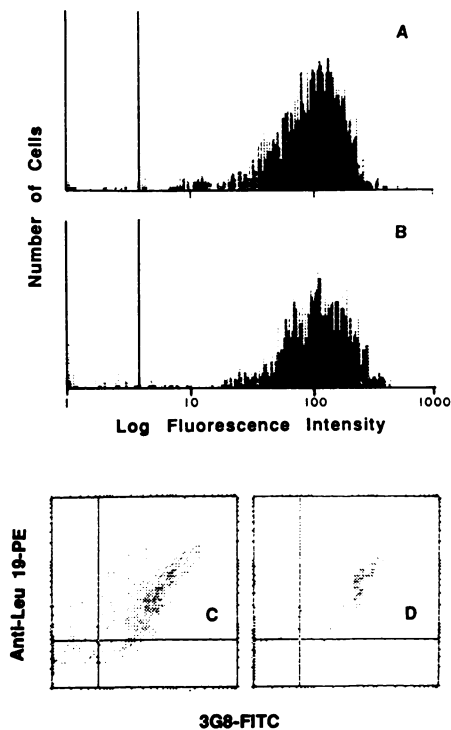**Log Fluorescence Intensity**

3G8-FITC

FIG. 5. Expression of NK cell surface antigens before and after PI-PLC digestion. Cells were double-stained with FITC-conjugated 3G8 and PE-conjugated anti-Leu-19 prior to (*C*) and following (*D*) PI-PLC digestion. Control NK cells were stained with anti-class I antibody W6/32 before (*A*) and after (*B*) PI-PLC digestion. Data shown are from one representative experiment.

library and all of these coded for a polypeptide-anchored form of CD16. From three independent cDNA clones isolated from a human PBL cDNA library, one encoded a polypeptide-anchored form, while two encoded a PI-G-anchored form. Although the number of cDNA clones analyzed is small, it is clear that the polypeptide-anchored form of CD16 appears to predominate in the lung.

CD16 has been shown to be polymorphic by serological (7) and biochemical (8) analyses. Polymorphism was also evident in our sequence data, as the molecules encoded by the HL12 and PBL14 cDNA clones differed by six amino acids in the extracytoplasmic domain. The cDNA clone reported by Simmons and Seed (11) predicts a protein that differs by two amino acids (positions 122 and 151) from the PBL14-encoded protein and six amino acids from the HL12-encoded protein. In addition, the PBL14-encoded protein may have an additional N-linked carbohydrate moiety. The sequence heterogeneity may reflect different alleles of a single gene or the presence of more than one CD16 gene in the human genome. [Our preliminary DNA hybridization experiments and those previously reported (11) did not reveal the presence of more than one gene.] It is also possible that the polypeptide-anchored and PI-G-anchored forms result from alternative RNA splicing mechanisms of a single gene transcript as demonstrated for neural cell adhesion molecule (23) and decay-accelerating factor (24).

The existence of a polypeptide-anchored form (as encoded by HL12) was supported by peptide sequencing data showing (1) arginine (predicted by HL12) as well as serine (predicted by PBL14) at position 36 of the purified protein and (2) only aspartic acid (predicted by HL12 but not by PBL14) at position 122. However, for reasons that are not clear, there were no clear indications of peptide heterogeneity at position 147 (peptide 8)

or position 176 (peptide 10) despite the fact that HL12 and PBL14 encode different amino acids at these positions.

Previous functional studies of CD16 have established this receptor's roles in removing immune complexes from circulation (3) and in mediating antibody-dependent cellular cytotoxicity (4, 5). The identification of PI-G-anchored CD16 molecules has raised questions of how such a receptor can mediate its various activities, including transmembrane signal transduction (9–11). The identification of a form containing a cytoplasmic domain may help to explain some of these activities. It should now be possible to address the specific functional and regulatory aspects of both the polypeptide-anchored and PI-G-anchored forms of CD16 and to investigate whether altered expression of these two forms correlates with various disease states.

1. Fleit, H. B., Wright, S. D. & Unkeless, J. C. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 3275–3279.
2. Perussia, B., Starr, S., Abraham, S., Fanning, V. & Trinchieri, G. (1983) *J. Immunol.* **130**, 2133–2141.
3. Clarkson, S. B., Kimberly, R. P., Valinsky, J. E., Witmer, M. D., Bussel, J. B., Nachman, R. L. & Unkeless, J. C. (1986) *J. Exp. Med.* **164**, 474–489.
4. Perussia, B., Acuto, O., Terhorst, C., Faust, J., Lazarus, R., Fanning, V. & Trinchieri, G. (1983) *J. Immunol.* **130**, 2142–2148.
5. Titus, J. A., Perez, P., Kaubisch, A., Garrido, M. A. & Segal, D. M. (1987) *J. Immunol.* **139**, 3153–3158.
6. Anasetti, C., Martin, P. J., June, C. H., Hellstrom, K. E., Ledbetter, J. A., Rabinovitch, P. S., Morishita, Y., Hellstrom, I. & Hansen, J. A. (1987) *J. Immunol.* **139**, 1772–1779.
7. Werner, G., von dem Borne, A. E. G., Bos, M. J. E., Tromp, J. F., van der Plas-van Dalen, C. M., Visser, F. J., Engelfriet, C. P. & Tetteroo, P. A. T. (1986) in *Leukocyte Typing*, eds. Reinherz, E. L., Haynes, B. F., Nadler, L. M. & Bernstein, I. D. (Springer, New York), Vol. 2, pp. 109–121.
8. Lanier, L. L., Ruitenberg, J. J. & Phillips, J. H. (1988) *J. Immunol.* **141**, 3478–3485.
9. Selvaraj, P., Rosse, W. F., Silber, R. & Springer, T. A. (1988) *Nature (London)* **333**, 565–567.
10. Huizinga, T. W. J., van der Schoot, C. E., Jost, C., Klaassen, R., Kleijer, M., von dem Borne, A. E. G. Kr., Roos, D. & Tetteroo, P. A. T. (1988) *Nature (London)* **333**, 667–669.
11. Simmons, D. & Seed, B. (1988) *Nature (London)* **333**, 568–570.
12. Portnoy, D. A., Erickson, A. H., Kochan, J., Ravetch, J. V. & Unkeless, J. C. (1986) *J. Biol. Chem.* **261**, 14697–14703.
13. Kinet, J.-P., Blank, U., Ra, C., White, K., Metzger, H. & Kochan, J. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 6483–6487.
14. Hewick, R. M., Hunkapillar, M. W., Hood, L. & Dreyer, W. J. (1981) *J. Biol. Chem.* **256**, 7990–7997.
15. Boyum, A. (1968) *Scand. J. Clin. Lab. Invest.* **21** Suppl. 97, 1.
16. Timonen, T. & Saksela, E. (1980) *J. Immunol. Methods* **36**, 285–291.
17. Low, M. G., Stiernberg, J., Waneck, G. L., Flavell, R. A. & Kincade, P. W. (1988) *J. Immunol. Methods* **113**, 101–111.
18. Ravetch, J. V., Luster, A. D., Weinshank, R., Kochan, J., Pavlovec, A., Portnoy, D. A., Hulmes, J., Pan, Y.-C. & Unkeless, J. C. (1986) *Science* **234**, 718–725.
19. Kochan, J., Pettine, L. F., Hakimi, J., Kishi, K. & Kinet, J.-P. (1988) *Nucleic Acids Res.* **16**, 3584.
20. Shimizu, A., Tepler, I., Benfey, P. N., Berenstein, E. H., Siraganian, R. P. & Leder, P. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 1907–1911.
21. Kinet, J.-P., Metzger, H., Hakimi, J. & Kochan, J. (1987) *Biochemistry* **26**, 4605–4610.
22. Ferguson, M. A. J. & Williams, A. F. (1988) *Annu. Rev. Biochem.* **57**, 285–320.
23. Cunningham, B. A., Hemperly, J. J., Murray, B. A., Prediger, E. A., Brackenbury, R. & Edelman, G. M. (1987) *Science* **236**, 799–806.
24. Caras, I. W., Davitz, M. A., Rhee, L., Weddell, G., Martin, D. W. & Nussenzweig, V. (1987) *Nature (London)* **235**, 545–549.