Engagement of p75/AIRM1 or CD33 inhibits the proliferation of normal or leukemic myeloid cells

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P75/AIRM1 is a recently identified surface molecule that belongs to the sialoadhesin family and displays homology with the myeloid cell antigen CD33. In lymphoid cells, p75/AIRM1 is confined to natural killer cells and mediates inhibition of their cytolytic activity. In this study, we show that p75/AIRM1 is also expressed by cells of the myelomonocytic cell lineage, in which it appears at a later stage as compared with CD33. *In vitro* proliferation and differentiation of cord blood-derived CD34$^+$ cells (induced by stem cell factor and granulocyte–macrophage colony-stimulating factor) were consistently inhibited by the addition of anti-p75/AIRM1 mAb. Engagement of CD33 led to inhibition in some experiments. A sharp decrease of cell proliferation/survival was detected in all three p75/AIRM1+ chronic myeloid leukemias analyzed when cultured in the presence of either anti-p75/AIRM1 or anti-CD33 mAbs. Thus, the present study suggests that p75/AIRM1 and CD33 may play a regulatory role in normal myelopoiesis and may be viewed as suitable target molecules to counteract the proliferation/survival of chronic myeloid leukemias.

It is well established that normal hemopoiesis is a multistep process in which lineage development occurs as a consequence of the ordered effect of a number of growth factors and of the expression of determined transcription factors (1, 2). This process is characterized by the sequential expression of surface markers, which allows recognition of various stages of cell differentiation and assessment of commitment to different lineages. For example, cells expressing CD34 include the pluripotent hemopoietic stem cells whereas CD33 is absent from these stem cells but appears on myelomonocytic precursors and continues to be expressed in both the myeloid and monocytic lineages while it is lost by mature granulocytes (3–5). Although CD33 represents a useful marker to distinguish myeloid from lymphoid leukemias, little is known of its function (6, 7). Being a member of the sialoadhesin family, it has been proposed to mediate cell-to-cell adhesion but it is unclear whether it actually plays any role in the process of myeloid cell differentiation (6).

Recently, we have identified and cloned p75/AIRM1, a novel molecule that functions as a potent inhibitory receptor in human natural killer (NK) cells. p75/AIRM1 is a type I transmembrane glycoprotein characterized by one IgV- and two IgC2-type domains. Remarkably, it was found to display homology with certain members of the sialoadhesin family, primarily with CD33 (8). In particular, both the IgV domain and the transmembrane region of p75/AIRM1 display a high degree of amino acid identity with CD33 molecule. In addition, both molecules are characterized by functional immunoreceptor tyrosine-based inhibition motifs (ITIMs) in their cytoplasmic tail (8, 9). Because the presence of ITIMs is a typical feature of different inhibitory receptors (10, 11), it is important to reinvestigate the role of CD33, especially with respect to its possible inhibitory function in hemopoietic cell proliferation and/or differentiation.

In this study, we show that, similar to CD33, p75/AIRM1 is also expressed by myelomonocytic cells. More importantly, engagement of p75/AIRM1 or CD33 led to a variable degree of inhibition of proliferation of normal myelomonocytic cell precursors and of chronic myeloid leukemias (CMLs). These data suggest a modulatory role of p75/AIRM1 and CD33 during myeloid differentiation and may offer clues toward novel approaches in the therapy of myeloid leukemias.

Materials and Methods

mAbs and Reagents. QA79 (IgG1) mAb was obtained by immunizing a 5-week-old BALB/c mouse with the NK clone LM5 (surface phenotype: CD3$^-$, CD16$^+$, CD56$^+$, NKp46$^+$, NKp44$^+$, p140$^+$, CD94/NKG2A+) as described previously (12). The following mAbs were produced in our lab: JT3A (IgG2a anti-CD3), KL247 (IgM anti-p46), BAB281 (IgG1 anti-p46), Z176 (IgG2b anti-p75/AIRM1), and E59–126 (IgG1 anti-IRp60). QA79 mAb, similarly to the previously described Z176 mAb, selectively reacted with CO7 cells transfected with the VR1012-AIRM1 construct (8). mAbs HPCA II (IgG1 anti-CD34) and Leu-M3 (IgG2b anti-CD14) were purchased from Becton Dickinson; mAb MY9 (anti-CD33 IgG2b) was purchased from Coulter. Purified mAb WM53 (IgG1 anti-CD33), sodium azide-free, and the FITC- and phycoerythrin (PE)-conjugated anti-isotype goat anti-mouse antibodies were purchased from Southern Biotechnology. The PE-conjugated anti-CD34 (IgG1) and FITC-conjugated anti-CD33 (IgG1) mAbs were purchased from Immunotech (Westbrook, ME). The affinity-purified anti-IgG (H+L) goat anti-mouse serum was purchased from ICN. Notably, the mAb-containing culture supernatants were endotoxin free. In addition, the WM53 anti-CD33 mAb was supplied as endotoxin-free.

The culture medium was Iscove's modified Dulbecco's medium supplemented with 1% L-glutamine (GIBCO/BRL) and antibiotic mixture (5 mg/ml penicillin, 5 mg/ml streptomycin, and 5 mg/ml neomycin stock solution; GIBCO), 10% of FCS (Sigma), and human recombinant granulocyte–macrophage colony-stimulating factor (GM-CSF) at the final concentration of 100 ng/ml and stem cell factor (SCF) at the final concentration of 50 ng/ml (PeproTech, Rocky Hill, NJ). Ficoll/Hypaque (F/H) density gradient was purchased from Sigma.

Isolation and Purification of CD34$^+$ Cell Precursors and CML-Derived Myeloid Cells. Cord blood samples from full-term newborns were collected upon informed consent of the mother at San Martino Hospital in Genoa, Italy. Mononucleated cells were isolated on F/H gradients, and CD34$^+$ were purified by immunomagnetic bead-positive selection by using the magnetic-activated cell sorter device (Mini-MACS) following the manufacturer's guide-

Abbreviations: NK, natural killer; ITIM, immunoreceptor tyrosine-based inhibition motif; CML, chronic myeloid leukemia; PE, phycoerythrin; GM-CSF, granulocyte–macrophage colony-stimulating factor; SCF, stem cell factor; F/H, Ficoll/Hypaque.

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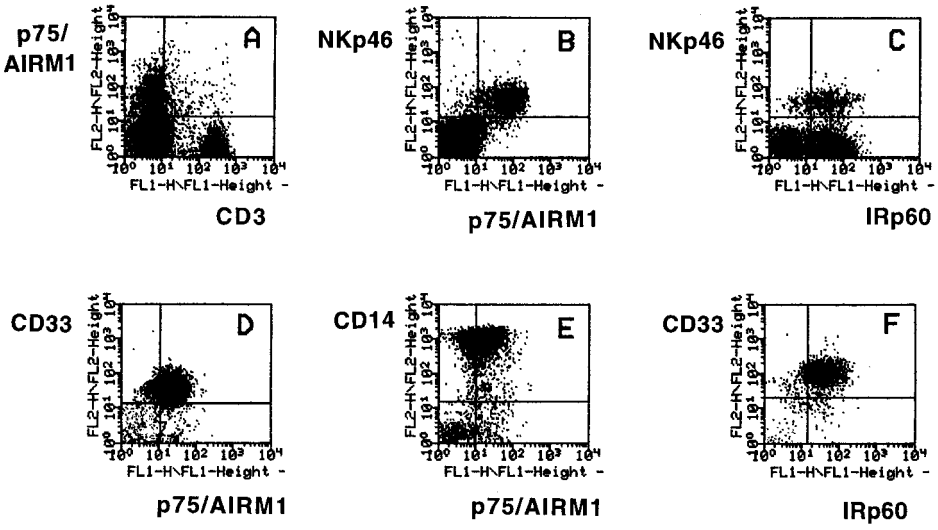

Fig. 1. Pattern of expression of p75/AIRM1 molecule in cord blood-derived lymphoid or myeloid cell populations. Cells were isolated from cord blood by the use of a F/H gradient and analyzed by two-color immunofluorescence and FACS analysis for the expression of p75/AIRM1 in combination with CD3, NKp46, CD14, or CD33 molecules. In *A–C*, analysis was performed on cells gated on lymphoid populations, whereas, in *D–F*, analysis was performed on cells gated on myeloid populations. *C* and *F* show lymphoid or myeloid cell populations stained with anti-IRp60 mAb in combination with anti-NKp46 or anti-CD33, respectively. The dot plots were divided into quadrants representing unstained cells (lower left), cells with only red fluorescence (upper left), cells with red and green fluorescence (upper right), and cells with only green fluorescence (lower right).

lines (Miltenyi Biotec, Auburn, CA). $CD34^+$ cells obtained after bead separation were >95%.

Peripheral blood samples from patients affected by CML were collected after informed consent at the San Martino Hospital, and mononucleated cells were isolated on F/H gradient. Cytogenetic analyses of bone marrow cells of patients 1, 2, 3, and 4 showed that 100% of metaphases carried t(9;22) (q 34; q 11); moreover, patient 2 showed an additional t(4; 10) (q 12; q 26).

Culture and Stimulation of $CD34^+$ Cells and Leukemic Cells. $CD34^+$ cord blood-derived cells were plated at the concentration of 2×10^4 cc/well in 96 flat-bottomed well plates and cultured in the presence of GM-CSF (100 ng/ml) + SCF (50 ng/ml) whereas CML cells were plated at the final concentration of 5×10^5 cc/well in 6 flat-bottomed well plates and cultured in the presence of GM-CSF alone (100 ng/ml). Both cell types, supplemented with the indicated growth factors, also were plated at the concentration of 2×10^4 cc/well in 96 flat-bottomed well plates coated with goat anti-mouse (10 μg/ml). Cells were cultured with saturating amounts of one or another of the following mAbs: QA79 (anti-p75), 50 μl of hybrid supernatant (at a final concentration of 5–10 μg/ml) or of purified mAb (10 μg/ml), purified anti-CD34 mAb (10 μg/ml), purified anti-CD33 mAb (10 μg/ml); E59–126 (anti-IRP 60), 50 μl of hybrid supernatant at a final concentration of 5–10 μg/ml.

At different time intervals (4, 6, and 8 days for cord blood-derived cells and 3, 4, 5, and 7 days for CML-derived cells), cells were harvested, counted, and analyzed for their surface phenotype.

Proliferation Assay. CML-derived myeloid cells cultured as described above for different time intervals (3, 4, 5, and 7 days) were pulsed with 1 μCi/well [^{3}H]thymidine (Amersham Pharmacia) 16 hr before harvesting. Cells were harvested by using a Titertek Cell Harvester 550. Radioactivity was measured in a scintillation β-counter (Beckman Coulter). All cultures were performed in triplicates.

Indirect Immunofluorescence and Cytofluorimetric Analysis. Before immunofluorescence analysis, fresh or cultured cells harvested at different time intervals were pretreated with human IgG (1 mg/ml) to block Fc receptors. $CD34^+$ also were treated with mouse-derived polyclonal Ig serum to eliminate cross-reactions with MACS beads. Cells then were analyzed by single and double immunofluorescence as described previously (13). Briefly, 5×10^4 cells were stained with the corresponding mAb followed by FITC- and PE-conjugated isotype-specific goat anti-mouse serum second reagent. Samples were analyzed on a flow cytometer (FACSort; Becton Dickinson) equipped with an argon ion laser exciting FITC at 488 nm and PE at 514 nm. Results are expressed as Log red fluorescence intensity (arbitrary units) vs. Log green fluorescence intensity (arbitrary units) or vs. cell number.

Results

p75/AIRM1 Molecule Is Expressed by Myelomonocytic Cells and Is Coexpressed with CD33. As reported previously, among lymphoid cells, p75/AIRM1 was expressed by most NK cells but was absent in B lymphocytes and in most T lymphocytes. More importantly, mAb-mediated crosslinking of p75/AIRM1 inhibited NK cell function (8). Here, we analyzed the expression of p75/AIRM1 on cells isolated from human cord blood or bone marrow. Thus, we assessed the expression of p75/AIRM1 in different leukocyte populations including myelomonocytic cells at different stages of maturation. Fig. 1 shows the reactivity of the anti-p75/AIRM1 QA79 mAb (IgG1) with cells isolated from cord blood (on F/H gradients). In agreement with previous data, FACS analysis of cells gated on lymphoid populations revealed that p75/AIRM1+ cells expressed NKp46 (a surface marker that precisely identifies the human NK cell population) but not CD3 (Fig. 1 *A* and *B*). In Fig. 1*C*, lymphoid cells are stained with the anti-IRp60 E59–126 mAb. IRp60 is a previously described surface molecule characterized by four ITIMs in the cytoplasmic tail that, similar to p75/AIRM1, functions as an inhibitory receptor in NK cells (14). In Fig. 1 *D* and *E*, the analysis of cells gated on myeloid populations revealed that p75/AIRM1 is expressed by most $CD33^+$ cells and by the majority of $CD14^+$ cells. Fig. 1*F* shows that IRP60 is coexpressed with CD33, thus indicating that this molecule is also expressed by myeloid cells. A similar pattern of reactivity has been observed in all 10 cord

blood samples analyzed. It should also be noted that, different from CD33, p75/AIRM1 is expressed by mature granulocytes (not shown). Analysis of adult bone marrow-derived samples confirmed the same pattern of expression of p75/AIRM1 in cells of the myelomonocytic cell lineage (not shown).

Analysis of the Surface Expression of p75/AIRM1 or CD33 Antigens in $CD34^+$ Cells Either Freshly Isolated or Cultured with SCF and GM-CSF. Analysis of purified $CD34^+$ cell populations revealed a variable pattern of expression of CD33. Thus, whereas in some experiments, isolated $CD34^+$ cells coexpressed CD33 in variable proportions, in others, no coexpression of CD33 could be detected. On the other hand, p75/AIRM1 was consistently absent in $CD34^+$ populations, thus suggesting that this surface molecule may be expressed later than CD33 during myeloid cell differentiation.

To determine the time course of expression of p75/AIRM1 in comparison with CD33, we selected those $CD34^+$ cell populations that lacked not only p75/AIRM1 but also CD33. These cells were cultured with SCF and GM-CSF and analyzed for the expression of the two markers at different time intervals. As shown in Fig. 2*A*, the starting $CD34^+$ cell population did not express either p75/AIRM1 or CD33. At day 4 of culture, a fraction of these cells expressed p75/AIRM1 and CD33. However, it is evident that the percentages of $CD33^+$ cells were higher than those of cells expressing p75/AIRM1. This was also true at the different time intervals analyzed including days 6, 10 (Fig. 2*A*), and 20 (not shown). Although not shown, in the same cultured cell populations, IRP60 displayed a pattern of surface expression similar to that of CD33. Analysis of marker expression at earlier culture intervals could not be performed because of the limited number of cells available. These data suggest that, although CD33 and p75/AIRM1 antigens were expressed by the same cell populations in fresh cord blood or bone marrow, their time of appearance during myeloid differentiation was different, the expression of CD33 preceding that of p75/AIRM1.

Effect of mAb-Mediated Crosslinking of p75/AIRM1 or CD33 on the Proliferation of Myelomonocytic Cell Precursors. Because engagement of p75/AIRM1 exerted a strong inhibitory effect on NK cell cytotoxicity, it could also induce inhibition of myeloid cell function. A similar possibility also could be envisaged for CD33 in view of its homology with p75/AIRM1 (including the presence of functional ITIMs in the cytoplasmic tail) (8, 9). Therefore, we analyzed whether addition of anti-p75/AIRM1 or anti-CD33 mAbs had any effect on the proliferation/differentiation of $CD34^+$ cells cultured with SCF and GM-CSF. Control cultures contained isotype-matched mAbs specific for CD34 or IRP60. Fig. 2*B* shows the data of five experiments in which both anti-p75/AIRM1 and anti-CD33 mAb have been used. Data represent the mean ($\pm$SE) of the percentages of viable cells recovered (at day 6) from cultures containing different mAbs vs. control cultures containing no mAb. It can be seen that, in cultures containing anti-CD34 or anti-IRP60 mAb, cells underwent proliferation similar to control cultures containing no mAb. On the other hand, a sharp inhibition of cell proliferation occurred in cultures containing anti-p75/AIRM1. Although anti-CD33 mAb did not appear to significantly affect cell recovery, it should be mentioned that in two of five experiments, $\approx$50% inhibition could be detected. Ten additional experiments performed with anti-p75/AIRM1 mAb confirmed the ability of this mAb to sharply inhibit proliferation of cultures derived from $CD34^+$ cell precursors (not shown).

Anti-CD33 or anti-p75/AIRM1 mAbs Inhibit Cell Proliferation of Chronic Myeloid Leukemias. Cells of CMLs presenting the typical t(9;22), isolated from four chemotherapy-free patients, were analyzed for the surface expression of p75/AIRM1, CD33, and IRP60. Leukemic cells from three of these patients were homogeneously $CD33^+$. In addition, they expressed, in variable proportions, p75/AIRM1 and IRP60 (Fig. 3 *Left*). The fourth patient expressed low levels of CD33 and virtually no p75/AIRM1. Leukemic cells from these patients were cultured in the presence of GM-CSF. Cells underwent variable degrees of proliferation as assessed by evaluating cell recovery at different culture intervals or by measuring the uptake of [^{3}H]thymidine. We analyzed whether addition of anti-p75/AIRM1, anti-CD33, or anti-IRP60 mAbs could affect the leukemic cell proliferation examined at different culture intervals. Cell proliferation in the absence of mAb assessed after 5 days of culture resulted in a 1.5- to 3.5-fold increase of the original cell input (not shown). Fig. 3 *Right* shows the effect of mAb addition on cell proliferation (at day 5) in the four patients. It is evident that both anti-p75/AIRM1 and anti-CD33 mAb exerted a strong inhibitory effect in the first three patients. Thus, the cell recovered after 5 days from cultures containing anti-p75/AIRM1 mAb ranged between 26% and 57% of cells recovered from control cultures containing no mAb. In cultures containing anti-CD33 mAb the recovered cells ranged between 24% and 55%. Again, anti-IRP60 mAb had no inhibitory effect. A similar inhibition was observed in parallel cultures in which cells were pulsed with [^{3}H]thymidine for 16 hr at day 5 (not shown).

Note that maximal inhibition mediated by anti-p75/AIRM1 was observed in patient 1 in which leukemic cells showed the highest expression of the antigen. Leukemic cells from patient 4 did not express p75/AIRM1, and, accordingly to this phenotype, no substantial effect on cell recovery could be detected.

Discussion

In this study we show that p75/AIRM1, a recently identified inhibitory receptor expressed by NK cells that displays homology with CD33, is also expressed by myelomonocytic cells during differentiation. More importantly, engagement of either p75/AIRM1 or CD33 may lead to a substantial inhibition of proliferation of both normal and leukemic myeloid cells.

P75/AIRM1 is a novel transmembrane glycoprotein that, in lymphoid cells, is expressed primarily by NK cells. Molecular cloning revealed homology with two members of the sialoadhesin family, namely, the myelomonocytic lineage CD33 and the placental CD33L1 antigens (4, 6, 8, 15). In this study we show that in cells of the myelomonocytic lineage isolated from the human cord blood, the surface expression of p75/AIRM1 appears to overlap largely with that of CD33. Different experimental evidence, however, would indicate that they are expressed at different stages of myeloid cell differentiation. Thus, (a fraction of) $CD34^+$ cells frequently coexpressed CD33, but not p75/AIRM1. In addition, during *in vitro* proliferation/maturation of purified $CD34^+$ cells, the percentages of cells expressing CD33 outnumbered those expressing p75/AIRM1. Finally, the CML cells analyzed expressed CD33, although in variable proportions, whereas p75/AIRM1 was expressed in lower percentages or, in one case, was missing. Taken together, these data support the concept that p75/AIRM1 may be expressed at a later stage of myeloid differentiation. Remarkably, similar to CD33, p75/AIRM1 was expressed on the majority of $CD14^+$ cells (monocytes). On the other hand, it was expressed by mature granulocytes that lack CD33 (not shown). These data suggest that p75/AIRM1 may represent a useful marker for further dissecting the normal myeloid differentiation process and, possibly, for a more accurate leukemic cell typing. Experiments to further analyze the expression of p75/AIRM1 during normal hemopoiesis as well as in different myeloid leukemias are in progress in our lab.

Regarding the various molecules belonging to the sialoadhesin family, beside their ability to bind sialic acid, limited information is available on their function. Apart from the recently demon-

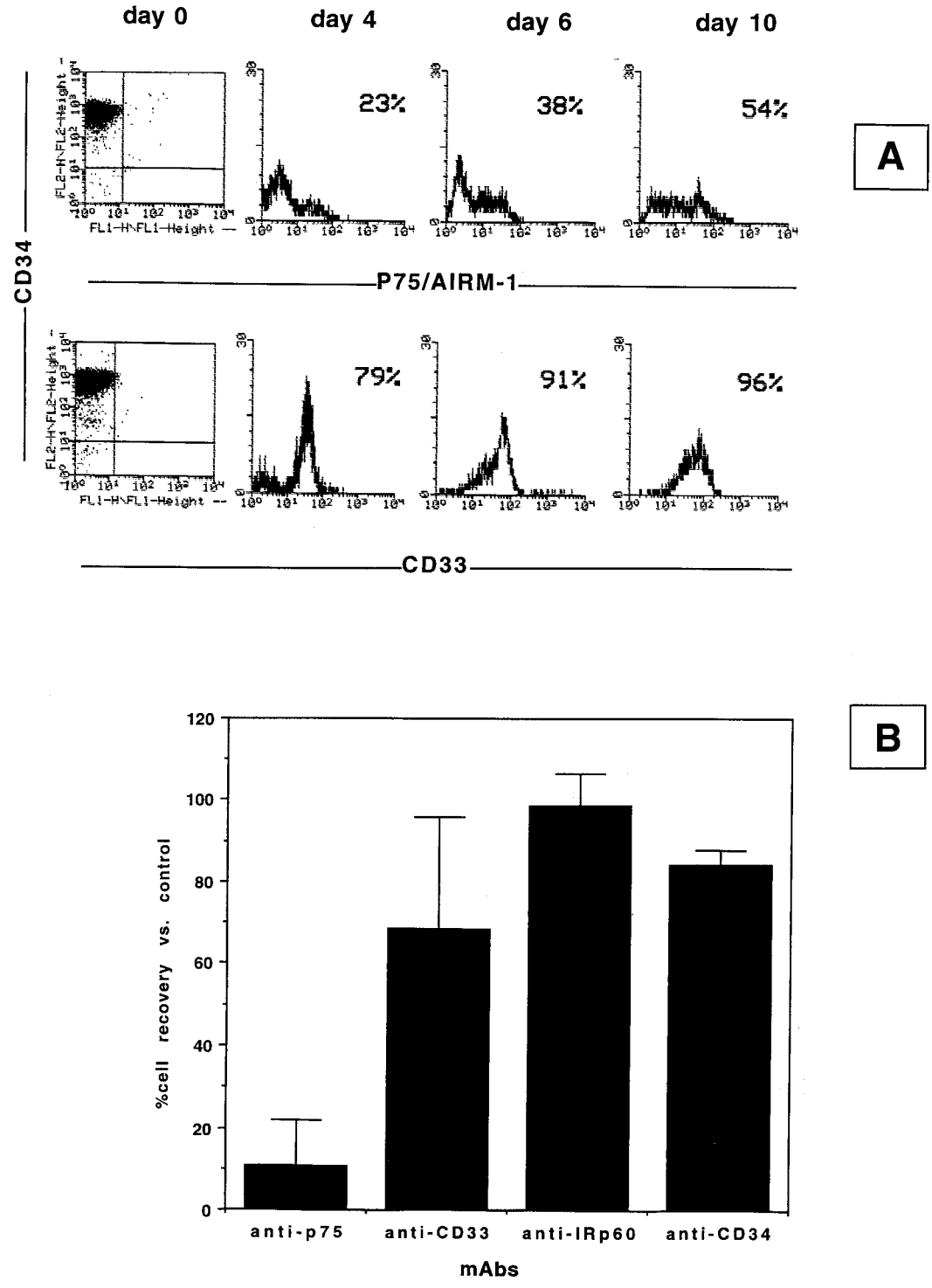

Fig. 2. Surface expression of p75/AIRM1 or CD33 antigens in fresh or cultured CD34$^+$ myeloid precursors isolated from cord blood and effect of anti-p75/AIRM1 or anti-CD33 mAb on their *in vitro* induced proliferation. A highly purified CD34$^+$ cell population that did not express either p75/AIRM1 or CD33 antigen (*A Left*) was cultured with SCF and GM-CSF. The surface expression of p75/AIRM1 or CD33 was analyzed at different culture intervals, as indicated. In *B*, CD34$^+$ populations isolated from different cord blood samples were analyzed for proliferation either in the absence of mAbs or in the presence of mAbs specific for p75/AIRM1, CD33, CD34, or IRp60 molecules. The data indicate the percentage of cell recovery (at day 6) in the indicated culture conditions vs. control culture (containing no mAb). Bars indicate the mean (±SE) of five independent experiments.

strated inhibitory effect of p75/AIRM1 on NK cell cytotoxicity, the only molecule of this family known to display inhibitory function is CD22, which is expressed on B cells and may down-regulate B cell receptor-mediated cell triggering (16, 17). Regarding CD33, no information was available on its possible function in myeloid cell differentiation. Clues that CD33 could exert a regulatory role in myeloid cell function have been provided by two recent observations: first, CD33 is homologous

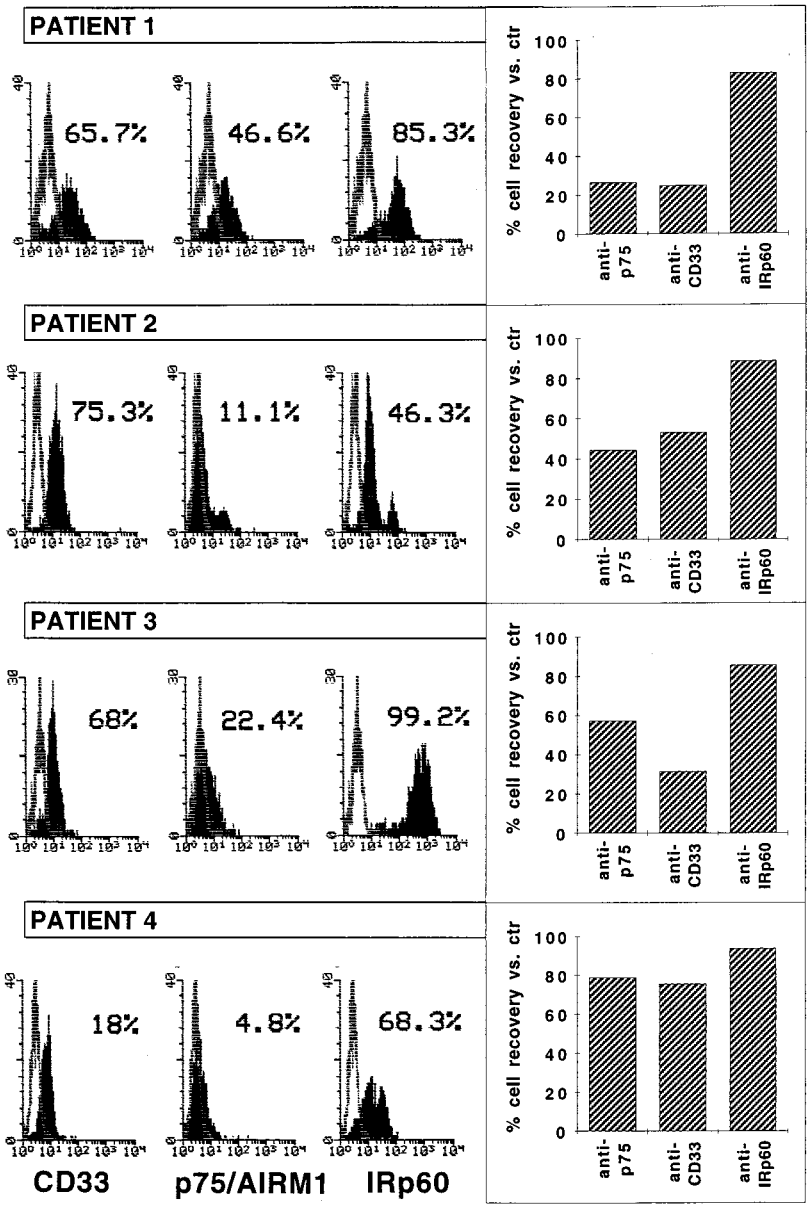

Fig. 3. Inhibitory effect of anti-p75/AIRM1 or anti-CD33 mAb on the *in vitro* proliferation of chronic myeloid leukemic cells. Cells were isolated on F/H density gradients from peripheral blood of four patients affected by chronic myeloid leukemias t(9;22) and analyzed for the surface expression of CD33, p75/AIRM1, and IRp60 by indirect immunofluorescence and FACS analysis (*Left*). Cells were cultured with GM-CSF either in the presence or in the absence of mAbs specific for p75/AIRM1, CD33, or IRP60 surface molecules. After 5 days, cells were harvested and viable cells were counted (*Right*); values indicate the percentages of viable cells recovered in each experimental condition vs. control cultures containing no mAb.

to p75/AIRM1, which functions as an inhibitory receptor (8); second, CD33 is characterized by ITIMs in its cytoplasmic tail that undergo phosphorylation upon mAb-mediated crosslinking (9). The present studies demonstrate directly that engagement of CD33 indeed may lead to some degree of inhibition of normal hemopoietic cell proliferation *in vitro*. Importantly, strong inhibition of normal myelopoiesis was detected consistently upon engagement of p75/AIRM1. Taken together, our data suggest that both CD33 and p75/AIRM1 may exert a regulatory role in normal hemopoiesis. Because they are expressed at different stages of myeloid differentiation, they may act at different stages of development. In addition, although both molecules bind ligands in a sialic-dependent fashion, it is conceivable that they may recognize different ligands. This would imply that CD33 and p75/AIRM1 may regulate normal myelopoiesis in response to different stimuli.

Remarkably, p75/AIRM1 inhibits both NK-mediated cytotoxicity and myelomonocytic cell proliferation. In contrast, IRP60, which exerted a similar inhibitory effect on NK cytotoxicity, had no effect on the proliferation of myeloid cells (14). This indicates that surface receptors with inhibitory activity on NK cytotoxicity and characterized by functional ITIMs cannot necessarily exert an inhibitory effect in myeloid cell proliferation.

Perhaps more importantly, both anti-p75/AIRM1 and anti-CD33 mAbs sharply inhibited the proliferation of three chronic myeloid leukemias expressing p75/AIRM1 and CD33 surface antigens. The molecular mechanism responsible for this effect remains to be determined. Because both p75/AIRM1 and CD33 can recruit and activate the hemopoietic phosphatase SHP-1, it is conceivable that SHP-1 may extinguish the activating signaling pathways leading to cell proliferation and survival. Another

possible mechanism might imply an interference with molecular regulators of the cell cycle. A still unexplained question is why anti-p75/AIRM1 mAb can exert a sharp inhibition of proliferation of cord blood-derived cell precursors although only a fraction of these cells expressed p75/AIRM1. It is possible that inhibition of cell growth may result both from a direct effect of the mAb on p75/AIRM1+ cells and from an indirect effect, possibly mediated by proteolytic enzymes released as a consequence of cell damage or death. Experiments aimed at the definition of the mechanism(s) involved in the p75/AIRM1- or CD33-mediated inhibition of myeloid cell proliferation and/or survival are in progress in our lab. Preliminary experiments would suggest that the engagement of CD33 in a leukemic cell line may act by inducing apoptosis. Whatever the mechanism, the present experiments indicate that signaling via inhibitory surface molecules that are expressed by myeloid cells may provide a powerful tool to counteract proliferation and/or survival of leukemic cells. A therapeutic approach based on the use of mAbs to CD33 or p75/AIRM1 may be particularly useful to eliminate residual leukemic cells after chemotherapy. Along this line, it is noteworthy that an anti-CD33 mAb recently has been applied successfully to the therapy of promyelocytic leukemias by Caron *et al.* (7, 18, 19). It is possible that the observed effect on leukemic cells in patients may result not only from their killing via phagocytosis or antibody-dependent, cell-mediated cytotoxicity, as suggested by the authors, but also by a direct inhibitory effect of anti-CD33 mAb on leukemic cells. Our present data also clearly imply that CD33 cannot be considered simply as a myeloid cell marker, but should be viewed as a signaling surface molecule possibly regulating myeloid cell growth and differentiation.

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1. Dexter, T. M. & Spooncer, E. (1987) *Annu. Rev. Immunol.* **3,** 423–441.
2. Ruscetti, F. W., Keller, J. & Longo, D. L. (1997) in *Harrison's: Principles of Internal Medicine*, eds. Fauci, A. S., Braunwald, E., Isselbacher, K. J., Wilson, J. D., Martin, J. B., Kasper, D. L., Hauser, S. L. & Longo, D. L. (McGraw–Hill, New York), 14th Ed., pp. 634–638.
3. Satterthwaite, A. B., Burn, T. C., Le Beau, M. M. & Tenen, D. G. (1992) *Genomics* **12,** 788–794.
4. Paiper, S. C., Ashmun, R. A. & Look, A. T. (1988) *Blood* **72,** 314–321.
5. Andrews, R. G., Singer, J. W. & Bernstein, I. D. (1989) *J. Exp. Med.* **169,** 1721–1731.
6. Freeman, S. D., Kelm, S., Barber, E. K. & Crocker, P. R. (1995) *Blood* **85,** 2005–2012.
7. Caron, P. C., Schwartz, M. A., Co, M. S., Queen, C., Finn, R. D., Graham, N. C., Divgi, C. R., Larson, S. M. & Scheinberg, D. A. (1994) *Cancer* **73,** 1049–1056.
8. Falco, M., Biassoni, R., Bottino, C., Vitale, M., Sivori, S., Augugliaro, R., Moretta, L. & Moretta, A. (1999) *J. Exp. Med.* **190,** 793–801.
9. Taylor, V. C., Buckley, C. D., Douglas, M., Cody, A. J., Simmons, D. L. & Freeman, S. D. (1999) **274,** 11505–11512.
10. Moretta, A., Bottino, C., Vitale, M., Pende, D., Biassoni, R., Mingari, M. C. & Moretta, L. (1996) *Annu. Rev. Immunol.* **14,** 619–648.
11. Long, E. O. (1999) *Annu. Rev. Immunol.* **17,** 875–904.
12. Moretta, A., Bottino, C., Pende, D., Tripodi, G., Tambussi, G., Viale, O., Orengo, A., Barbaresi, M., Merli, A., Ciccone, E. & Moretta, L. (1990) *J. Exp. Med.* **172,** 1589–1598.
13. Mingari, M. C., Poggi, A., Biassoni, R., Bellomo, R., Ciccone, E., Pella, N., Morelli, L., Verdiani, S., Moretta, A. & Moretta, L. (1991) *J. Exp. Med.* **174,** 21–26.
14. Cantoni, C., Bottino, C., Augugliaro, R., Morelli, L., Marcenaro, E., Castriconi, R., Vitale, M., Pende, D., Sivori, S., Millo, R., *et al.* (2000) *Eur. J. Immunol.*, in press.
15. Takei, Y., Sasaki, S., Fujiwara, T., Takahashi, E., Muto, T. & Nakamura, Y. (1997) *Cytogenet. Cell. Genet.* **78,** 295–300.
16. Tedder, T. F., Tuscano, J., Sato, S. & Kehrl, J. H. (1997) *Annu. Rev. Immunol.* **15,** 481–504.
17. Law, C. L., Sidorenko, S. P., Chandran, K. A., Zhao, Z., Shen, S. H., Fisher, E. H. & Clark, E. A. (1996) *J. Exp. Med.* **183,** 547–560.
18. Caron, P. C., Lai, L. T. & Scheinberg, D. A. (1995) *Clin. Cancer Res.* **1,** 63–70.
19. Caron, P. C., Dumont, L. & Scheinberg, D. A. (1998) *Clin. Cancer Res.* **4,** 1421–1428.