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30. Synthetic oligoribonucleotides, containing IBS1 and IBS2 sequences (5′-GGGAACAAAGGTTAATTGTTGTGTTTATGGACAGA; 35-mer) and pseudoligated substrate RNAs that comprised an internal IBS1 sequence (5′-GGGAACAAAGGAAGACAGA: pUUUUUUUU; 27-mer) were synthesized and purified as described (*20*). The sequence N_{29}-GACAGA:*pCp was prepared as described (*20*). Labeled reaction products that corresponded in size to a 26-mer (*pN_{13}GACAG:pUUUUUUUU) up to a 30-mer (*pN_{13}GACAGpA_4:$pA_3pA_2pA_1$pUUUUUUUU) were gel extracted and tagged by virtue of their 3′ terminal OH group to the 5′ phosphate group of the Eco RI site of plasmid BS/bl1 (*22*) DNA in the presence T4 RNA ligase (*20*). The 3′ OH group of the Eco RI site was used to initiate the complementary DNA (cDNA) synthesis of the covalently ligated RNA products. Amplification with the polymerase chain reaction was performed with a *bl1* internal oligonucleotide (5′-GATTAATGTGAAAGCATGCTAACTTC, nucleotides 635 to 660 on the plus strand of *bl1* and biotinylated on the 5′ end) in conjunction with an oligonucleotide partially complementary to the 5′ part of the cDNA sequence (5′-AAATCTGGTAACGGGAACAAAGGAAGAC). Direct solid-phase sequencing was performed as described [T. Hultman, S. Staahl, E. Hornes, M. Uhlen, *Nucleic Acids Res.* **17**, 4937 (1989)].
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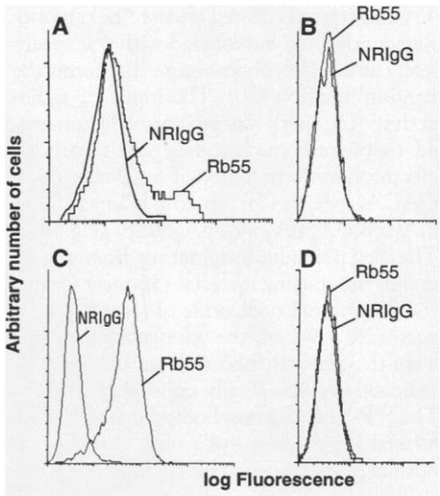

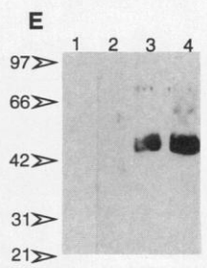

Fig. 1. The binding of Rb55 to a 45-kD molecule expressed on CD23-positive but not on CD23-negative cells. Flow cytometric analysis of (**A**) COS cells transfected with full-length human CD23 (*5*), (**B**) COS cells mock-transfected, (**C**) CD23-positive lymphoblastoid RPMI 8866 cell line, and (**D**) CD23-negative Burkitt lymphoma Daudi cell line. Cells were stained with affinity-purified Rb55 (*7*) or normal rabbit IgG (NRIgG) (both at 10 μg/ml) and fluorescein isothiocyanate (FITC)–conjugated goat antibodies to rabbit IgG (1 μg/ml) (Silenus, Victoria, Australia). Cells (5000) were analyzed on a FACScan (Becton Dickinson, Erembodeggem, Belgium). (**E**) Protein immunoblot analysis of CD23-positive and -negative cells. Cell lysates were subjected to SDS-PAGE. We transferred the separated proteins to a nitrocellulose filter and blotted them with Rb55 and horseradish peroxidase (HRP)–labeled goat antibodies to rabbit IgG (Sigma, St. Louis, Missouri). The mass standards are indicated at the left in kilodaltons. Lane 1, mock-transfected COS cells; lane 2, Daudi cells; lane 3, CD23-transfected COS cells; and lane 4, RPMI 8866 cells.

Inhibition of an in Vivo Antigen-Specific IgE Response by Antibodies to CD23

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Immunoglobulin E (IgE) mediates many allergic responses. CD23 is a 45-kilodalton type II transmembrane glycoprotein expressed in many cell types. It is a low-affinity IgE receptor and interacts specifically with CD21, thereby modulating IgE production by B lymphocytes in vitro. In an in vivo model of an allergen-specific IgE response, administration of a rabbit polyclonal antibody to recombinant human truncated CD23 resulted in up to 90 percent inhibition of ovalbumin-specific IgE synthesis. Both Fabs and intact IgG inhibited IgE production in vitro and in vivo. Thus, CD23 participates in the regulation of IgE synthesis in vivo and so could be important in allergic disease.

In vitro studies with some antibodies to CD23 (*1*, *2*) or soluble CD23 fragments (*3*) have implicated this molecule in the regulation of IgE synthesis. To investigate the importance of CD23 in the production of IgE in vivo [CD23 is a low-affinity receptor for IgE (*4*)], we used a rabbit polyclonal antibody (Rb55) that was raised to a truncated form of the human CD23 molecule. The immunogen, a 25-kD form of CD23, corresponded to amino acids 150 to 321 of full-length CD23 (*5*) and was produced in *Escherichia coli*. It was purified from a washed pellet of *E. coli* by ion-exchange and gel filtration chromatography and has the expected NH_2-terminal sequence (*6*). This 25-kD CD23 antigen was injected into a rabbit, and the resulting antiserum (Rb55) (*7*) tested positive in both an enzyme-linked immunosorbent assay (ELISA) and protein immunoblot with purified recombinant human CD23. An IgG fraction, isolated by protein A–Sepharose affinity chromatography, recognized recombinant CD23 expressed on COS cells transfected with cDNA encoding the full-length molecule as well as native CD23, as expressed on the Epstein-Barr virus (EBV)–transformed lymphoblastoid cell line RPMI 8866. Both CD23s were 45 kD (Fig. 1). Affinity-purified Rb55 IgG molecules and Fabs of Rb55 (*7*) were found to inhibit interleukin-4 (IL-4)–induced IgE production by human mononuclear cells in vitro in an isotype-specific

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manner (Fig. 2A), as do certain other monoclonal antibodies to CD23 (*1, 2*).

The truncated CD23 we used to generate Rb55 spanned the lectin homology domain of the molecule, a region conserved between mouse and human CD23 (*5, 8*). We therefore tested whether Rb55 recognized the corresponding molecule in the rat. The Rb55 reacted with splenic B cells from rats that were stimulated by IgE in vivo (Fig. 2B), a process that induces a low-affinity receptor for IgE (*9*). The molecule on rat splenic B cells that was recognized by Rb55 was purified on a rat IgE affinity column. We analyzed the eluate by SDS–polyacrylamide gel electrophoresis (SDS-PAGE) and protein immunoblotting and identified an Rb55-binding molecule of approximately 45 kD (Fig. 2C), a mass equivalent to that of the CD23 molecule recognized by Rb55 on the human cell line RPMI 8866 (Fig. 1). The 45-kD immunopurified rat molecule was reactive with four different antibodies to human CD23 peptides that spanned the lectin domain of the human CD23 molecule (*10*), suggesting that this is the rat homolog of human CD23.

We cloned rat CD23 by reverse transcriptase–polymerase chain reaction (RT-PCR) from rat splenic B cell and rat RBL-2H3 cell libraries, using oligonucleotide primers conserved between mouse (*8*) and human (*5*) sequences. The protein sequence homologies between rat and human CD23 and between rat and mouse are 71 and 92%, respectively (GenBank accession number is X73579). The high degree of CD23 homology may explain the cross-reactivity of Rb55 with rat and mouse CD23. The Rb55 reacted equally with a peptide derived from the rat CD23 sequence (residues 249 to 260) and with a peptide from the human CD23 sequence (residues 248 to 259) (Fig. 2D). Both sequences are inside the lectin domain. In contrast to some mAbs to CD23 (*11*), Rb55 did not bind to vimentin (*12*). These data demonstrate that Rb55 reacts with rat CD23.

To examine whether this antibody had an effect on IgE synthesis in vivo, we injected rats with the potent stimulator of IgE, pertussis toxin, and the protein antigen ovalbumin to generate anti-ovalbumin–specific IgE (*13*). When Rb55 was injected during the induction of an IgE response by ovalbumin and pertussis toxin, it inhibited the increase in serum polyclonal IgE by almost 90%. This occurred with both total and affinity-purified IgG fractions of Rb55. The control rabbit IgG preparation had no effect (Table 1). Maximum inhibition was measured at day 16. Not only was polyclonal IgE inhibited, but the development of an antigen-specific IgE re-

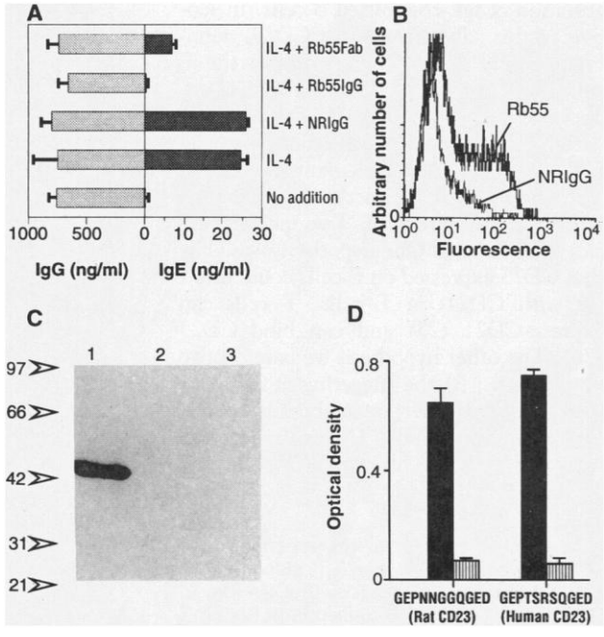

Fig. 2. Immunoreactivity and in vitro bioactivity of Rb55. (**A**) Class-specific inhibition of IgE production by Rb55 in vitro. Human peripheral blood mononuclear cells were incubated for 14 days alone or with recombinant IL-4 (100 U/ml) (Amersham, Buckinghamshire, United Kingdom). Affinity-purified IgG or Fabs of Rb55 (*7*) and NRIgG (both at 10 μg/ml) were used in the presence of IL-4. We measured Ig by specific ELISA (*2*). (**B**) Flow cytometric analysis of rat splenic B cells from Lou/c rats treated in vivo with IgE (*9*). Cells were stained with affinity-purified Rb55 (*7*) or NRIgG (both at 10 μg/ml) and FITC-conjugated goat antibodies to rabbit IgG (1 μg/ml) (Silenus). We analyzed cells (5000) on a FACScan (Becton Dickinson). (**C**) Protein immunoblot analysis of rat splenic B cell lysate immunoaffinity-purified on a rat IgE antibody column. Splenic B cell lysate from in vivo IgE-treated Lou/c rats (*9*) was separated on an Affi-gel 10 column (Bio-Rad, Richmond, California) coupled with rat IgE antibody, IR 162 (5 mg of antibody per milliliter of resin, according to manufacturer's instructions). Bound material was eluted with 3 M potassium thiocyanate (KSCN) and subjected to SDS-PAGE. Separated proteins were transferred to a nitrocellulose filter and blotted with Rb55 and HRP-labeled goat antibodies to rabbit IgG (Sigma). The mass standards are indicated at the left in kilodaltons. Lane 1, eluted material from rat IgE column; lane 2, flow-through from rat IgE column; and lane 3, eluted material from bovine serum albumin (BSA) (Sigma) column. (**D**) Reactivity of Rb55 with rat and human CD23–derived peptides determined by ELISA. Peptides (*29*) inside the lectin domain of rat and human CD23 sequences were coated to 96-well microtiter plates (Dynatech, Embrach, Switzerland) at 10 μg/ml. After saturating them with 3% BSA in phosphate-buffered saline, we incubated the plates with Rb55 (black bar) or NRIgG (striped bar) at 10 μg/ml and then with a peroxidase-coupled goat antibody to rabbit Ig (diluted 1:1000) (Sigma). The plates were revealed by *O*-phenylenediamine and H_2O_2 (Sigma). Optical densities read at 492 nm ± SD values of three experiments tested in duplicates are presented.

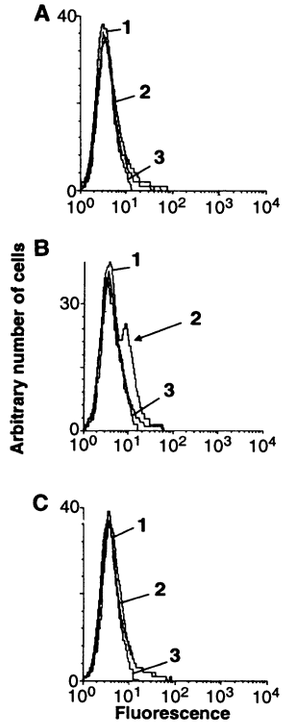

Fig. 3. Correlation between IgE concentrations in vivo and low-affinity IgE receptor expression. We tested rats from different groups (see Table 1) individually on day 16 for serum IgE concentration by ELISA (as described in Table 1) and for expression of low-affinity receptors on splenic cells by flow cytometry. Only fluorescence profiles from one rat are shown. (**A**) Rats ($n = 2$) received no stimulus and no Rb55 treatment, IgE concentration at day 16 (0.5 μg per milliliter of serum); (**B**) rats ($n = 2$) were stimulated with pertussis toxin plus ovalbumin and were treated with NRIgG, IgE concentration at day 16 (47 μg/ml); and (**C**) rats ($n = 3$) were stimulated with pertussis toxin plus ovalbumin and treated with Rb55, IgE concentration at day 16 (3.2 μg/ml). Profile 1 represents splenic B cells incubated with streptavidin-FITC (Becton Dickinson), profile 2 represents cells incubated with biotinylated rat IgE followed by streptavidin-FITC, and profile 3 represents cells incubated with FITC-labeled goat antibodies to rabbit. In (B) the IgE receptor is evident (arrow).

sponse was also inhibited by over 90% by Rb55 (Table 1). This inhibition was class-specific in that animals injected with Rb55 showed 115% and 92% of total and ovalbumin-specific polyclonal IgG and IgG1, IgG2a, IgG2b, and IgG2c subclasses (*14*), respectively, when compared to the control rats that were injected with control rabbit IgG (Table 1). Consistent with the in vitro data (Fig. 2B), Rb55 Fabs were also able to inhibit in an isotype-specific manner polyclonal (85% inhibition) as well as antigen-specific (86% inhibition) IgE production (Table 1).

We tested rats for the expression of low-affinity IgE receptors on their B cells by injecting the rats with pertussis toxin and ovalbumin to induce an IgE response. Purified spleen mononuclear cells from immunized rats, but not from control rats, expressed IgE receptors. In rats treated with Rb55, low-affinity IgE receptor expression was absent (Fig. 3). The absence of IgE binding to spleen cells of Rb55-treated rats was not due to the presence of Rb55 still bound to the cell surface, because Rb55 could not be detected on those cells (Fig. 3). The inhibition of low-affinity receptor expression correlated with the inhibition of IgE production described above (Fig. 3). This inhibition could be the result of a secondarily decreased IgE production. Because IgE stabilizes membrane CD23 by preventing its degradation (*15*), decreased IgE synthesis could result in decreased CD23 expression.

These observations show that CD23 is involved in an antigen-specific IgE response. In mice and humans, it has been shown that IgE responses, both in vivo (*16*) and in vitro (*1, 17*), are critically dependent on the T cell cytokine IL-4, which is believed to act by inducing the class switching of B cells so that they become IgE-committed (*18*). Because IL-4 is a very potent inducer of CD23 on lymphocytes (*19, 20*), it is likely that this CD23 induction is also important to the development of an IgE response. Our present results support this hypothesis.

At least two possibilities exist for the mechanism of action of Rb55 and for the role of CD23 in IgE synthesis: either Rb55 sends an intracellular signal through CD23 or blocks CD23 function during B cell–T cell interaction. First, the binding of Rb55 or IgE to CD23 on the B cell could trigger a negative feedback signal that would occur once IgE concentrations had increased above a certain threshold. But Fabs of Rb55 were as potent as Rb55 intact IgG in inhibiting IgE production in vitro and in vivo. Moreover, Fabs of monoclonal antibodies to CD23, in contrast to intact IgG, cannot transduce a signal through CD23 (*21*). These data suggest that neither cross-linking of CD23 nor negative signaling through CD23 is involved in the observed inhibition of IgE production.

Alternatively, CD23 may be acting as an adhesion molecule in cell-cell interactions that are necessary for complete differentiation of IgE-committed B cells. In support of this adhesive role for CD23, membrane-bound CD23 is involved in the B cell–T cell interaction (*22*), which is important in IgE synthesis (*23*); it interacts with CD21 (*24*), a molecule that is also the EBV receptor and complement receptor-2. The CD23-CD21 interaction regulates IgE production in vitro (*24*). Two mechanisms can be envisaged. One hypothesis would be that CD23 expressed on B cells could interact with CD21 on T cells. T cells can express CD21 (*25*) and can bind CD23 (*26*). The other hypothesis we base on two observations: (i) the triggering of CD21 by antibodies to CD21, recombinant soluble CD23 (*27*), and EBV (*28*) enhances IgE production even in absence of T cells, and (ii) IL-4 and specific allergen induce CD23 on T cells (*20*). On the basis of these observations, we speculate that in rats immunized with pertussis and ovalbumin, T cell–associated CD23 interacts with B cell–expressed CD21, leading to an increase in IgE production in vivo. In conclusion, these results demonstrate that CD23 plays a central role in the control of IgE production in vivo.

Table 1. Effect of Rb55 on the induction of an antigen-specific and polyclonal IgE response in vivo in the rat. Groups of six rats (250-g Lou/c rats) were injected with pertussis toxin (PT) alone (500 ng per rat) (day 0), PT (day 0) and ovalbumin (OVA) (1 mg per rat) (day 2) or PT (day 0), OVA (day 2) and normal rabbit IgG (NRIgG), Rb55IgG Rb55Fab (5 mg per rat), or affinity-purified (a-p) Rb55IgG (1 mg per rat) (*7*) (days 0, 2, and 9). All injections were intraperitoneal, except PT which was given intravenously. These experiments were performed in accordance with the Office Vétérinaire Cantonal guidelines. The combination of PT + OVA gave values for OVA-specific IgE and IgG of 26 ± 3 μg/ml and 150 ± 25 μg/ml, respectively. Neither PT nor OVA alone gave values above group 1 values. Sera were collected on day 16, and total and OVA-specific IgE and IgG were measured by ELISA. Values are mean ± 1 SD.

Group	Stimulus	Antibody	IgE		IgG	
			Total (μg/ml)	OVA-specific (μg/ml)	Total (mg/ml)	OVA-specific (μg/ml)
1	None		<0.1	<0.2	1.9 ± 0.5	<0.2
2	PT + OVA	NRIgG	28.0 ± 6	7.0 ± 0.3	1.8 ± 0.6	130 ± 15
3	PT + OVA	Rb55IgG	3.2 ± 2	0.4 ± 0.1	2.1 ± 0.4	120 ± 20
4	PT + OVA	Rb55Fab	4.1 ± 1	1.0 ± 0.8	2.3 ± 0.6	131 ± 17
5	PT + OVA	Rb55a-p	2.5 ± 2	0.2 ± 0.2	2.0 ± 0.7	127 ± 32

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6. Full-length CD23 cDNA sequence (*5*) was cut with Sau IIIA and Rsa I restriction enzymes. The fragment starting at position 714 and ending at position 1246 was then ligated to a synthetic oligonucleotide spanning the region 660 to 713. This construct was inserted into the plasmid pL as described [B. Allet *et al.*, *Gene* **65**, 259 (1988)] and expressed in *E. coli* strain B. Recombinant CD23 was produced as inclusion bodies. We broke cells by French pressure cell in tris-HCl (Sigma) buffer (pH 7.8). The insoluble material was washed with Triton X-100 (Fluka Chemika, Buchs, Switzerland) and then extracted with 6 M guanidinium hydrochloride (GuHCl) (Fluka Chemika) and chromatographed on Sephacryl S200-HR (Pharmacia, Uppsala, Sweden) in 4 M GuHCl. The 25-kD CD23-containing fractions were pooled, dialyzed against 6 M urea (Fluka Chemika) in acetate buffer (pH 4.7), and loaded on a Fast S column (Pharmacia). The material was 80% pure at this stage.
7. Injections of purified recombinant 25-kD CD23 (100 μg) were administered to a rabbit subcutaneously every 2 months, in accordance with the Office Vétérinaire Cantonal guidelines. The initial injection was in complete Freund's adjuvant with subsequent injections in incomplete Freund's adjuvant. Once a titer was established, we collected plasma every month. Total IgG and affinity-purified IgG fractions were prepared by passage over a protein A column (Bio-Rad, Richmond, CA) and a CD23–Affi-gel 15 column (Bio-Rad), respectively, and eluted by 3 M KSCN. The pooled eluted fractions were then dialyzed overnight against phosphate-buffered saline and concentrated by ultrafiltration (PM 10, Amicon, Beverley, MA). We generated Fabs of antibodies to CD23 by treating the antibodies with papain (Pierce, Oud Beijerland, Netherlands) according to manufacturer's instructions.
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29. Abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
30. We thank N. Aubonney, S. de Vevey, C. Losberger, L. Potier, and C. Siegfried for technical assistance; G. Mazzei for sequence analysis; H. Kikutani for the gift of human CD23 cDNA; M. Leeman-Husler for the graphics; and J. Knowles, K. Hardy, and J.-J. Mermod for discussions and support. The study in Brussels has been supported by the Fond de la Recherche Scientifique Médicale (Belgium).

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Fusion Between Transcription Factor CBFβ/PEBP2β and a Myosin Heavy Chain in Acute Myeloid Leukemia

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The pericentric inversion of chromosome 16 [inv(16)(p13q22)] is a characteristic karyotypic abnormality associated with acute myeloid leukemia, most commonly of the M4Eo subtype. The 16p and 16q breakpoints were pinpointed by yeast artificial chromosome and cosmid cloning, and the two genes involved in this inversion were identified. On 16q the inversion occurred near the end of the coding region for CBFβ, also known as PEBP2β, a subunit of a heterodimeric transcription factor regulating genes expressed in T cells; on 16p a smooth muscle myosin heavy chain (SMMHC) gene (*MYH11*) was interrupted. In six of six inv(16) patient samples tested, an in-frame fusion messenger RNA was demonstrated that connected the first 165 amino acids of CBFβ with the tail region of SMMHC. The repeated coiled coil of SMMHC may result in dimerization of the CBFβ fusion protein, which in turn would lead to alterations in transcriptional regulation and contribute to leukemic transformation.

Molecular analysis of specific chromosome abnormalities observed in tumor cells has led to the discovery of different genetic events leading to tumorigenesis. In leukemias, at least two mechanisms have been identified for the deregulation of cellular proto-oncogenes by chromosome rearrangements: (i) juxtaposition of a cellular proto-oncogene to the regulatory elements of a tissue-specific gene, particularly the immunoglobulin and T cell receptor genes, that leads to the inappropriate expression of the oncogene (*1*) and (ii) gene fusion at the junction of a translocation that generates a chimeric mRNA and a protein with transforming properties (*2*).

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A characteristic chromosome 16 pericentric inversion, inv(16)(p13q22), has been found in almost all patients with the M4Eo subtype, which constitutes about 8% of acute myeloid leukemia (AML) patients (*3*). Given the absence of other karyotypic abnormalities in many of these patients, a pathogenic relationship between inv(16) and AML M4Eo has been suggested (*3–5*).

Yeast artificial chromosomes (YACs) identified as containing the p arm inversion breakpoint (*6*) were used to screen the Los Alamos chromosome 16 cosmid library (*7*). To find which cosmids contained the breakpoint, we used clones so isolated as probes for fluorescence in situ hybridization (FISH). One of the cosmid clones, 16C3, generated single, discrete fluorescent signals on 16p from normal lymphoblastoid cells and one signal on each arm of the inverted 16 from the cell line ME-1, which was derived from a patient with AML M4Eo and inv(16) (*8*) (Fig. 1A). These data indicate that the 16p breakpoint lies within this cosmid (Fig. 2A). We confirmed this finding with peripheral leukemic cells from three additional patients who had inv(16) (*9*). Moreover, when a 1.2-kb Eco RI repeat-free fragment from cosmid 16C3 (named 16C3e) was used as a probe, rearranged fragments were detected by Southern (DNA) blot hybridization in multiple patients as well as when the DNA was

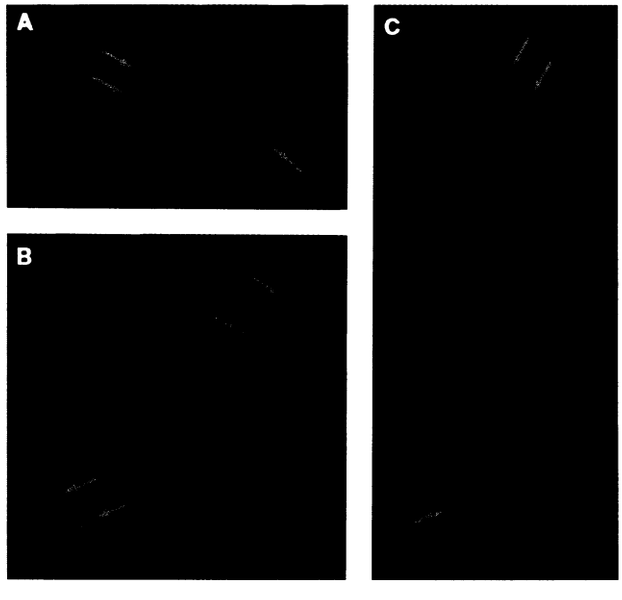

Fig. 1. Results from FISH with cosmids at the inv(16) breakpoint. (**A**) Cosmid 16C3 as a probe on a partial metaphase from the inv(16) cell line ME-1 (*8*). (**B**) A partial metaphase of a normal human lymphocyte probed with cosmid CC19. (**C**) Cosmids LA2-2 and LA4-1 on a partial metaphase from an inv(16) patient. We performed FISH as previously described (*6*).