The EMBO Journal vol.9 no.6 pp.1823 – 1830, 1990

Establishment of an interleukin-5-dependent subclone from an interleukin-3-dependent murine hemopoietic progenitor cell line, LyD9, and its malignant transformation by autocrine secretion of interleukin-5

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Communicated by J.Tooze

An interleukin-5 (IL-5)-dependent subclone, K-5, was established from an IL-3-dependent murine hemopoietic progenitor cell line by co-culturing with bone marrow stroma cells. K-5 cells were induced to differentiate into myeloid lineage cells by co-culturing with cloned PA6 stroma cells. By co-culturing with another cloned stroma cell (ST-2s10), K-5 cells gave rise to a factor-independent transformant cell line LT-5 which proliferated in an autocrine manner by secretion of IL-5 and produced tumors in nude mice. Molecular cloning of the IL-5 gene of LT-5 cells and the nucleotide sequencing of its 5′ flanking region indicate that a transposition of an intracisternal A-particle (IAP) element to the 5′ flanking region of the IL-5 gene is responsible for the constitutive expression of IL-5 mRNA of an aberrant size in LT-5 cells.

Key words: autocrine growth/cell differentiation/interleukin-5/intracisternal A-particle

Introduction

All hemopoietic cells, including T and B lymphocytes are considered to be derived from a common precursor cell—the stem cell—in the bone marrow (Dick *et al.*, 1985; Keller *et al.*, 1985). It has been shown that various growth factors are involved in differentiation of stem cells into blood cells. Establishment of many factor-dependent progenitor cell lines have provided useful experimental systems for studying molecular mechanisms of blood cell differentiation.

One such cell line, LyD9, which requires interleukin-3 (IL-3) for its growth and has the immunoglobulin gene in the germ-line context, is capable of generating mature B cells *in vivo* (Palacios and Steinmetz, 1985; Palacios *et al.*, 1987). Subsequently, we showed that LyD9 cells could differentiate into myeloid cells as well as mature B cells by co-culturing with stroma cells *in vitro* (Kinashi *et al.*, 1988, 1989). During the course of these studies we established an IL-4-dependent derivative (K-4) of LyD9, which seems to be an intermediate of *in vitro* differentiation into myeloid cells as well as B lymphocytes. Other derivatives (K-GM and LS-1) of LyD9 that respond to colony-stimulating factors (CSFs) seem to be committed to the myeloid lineage (Kinashi *et al.*, 1989).

In this report we describe another derivative (K-5) of LyD9 that requires IL-5 for its growth. K-5 cells were induced to differentiate into myeloid cells by co-culturing with a stroma cell line, PA6. In addition, a spontaneous transformant cell line of K-5 was obtained by the co-culture with another stroma cell line ST2. The transformant grew in an autocrine manner by secretion of IL-5, which appeared to be caused by transposition of an intracisternal A-particle (IAP) element to the 5′ flanking region of the IL-5 gene.

Results

K-5 cells differentiate into myeloid cells

LyD9 cells differentiate into myeloid as well as lymphoid cells when co-cultured with the primary culture of bone marrow stroma cells (Kinashi *et al.*, 1989). Such induced LyD9 cells also gave rise to progeny clones like K-4 and K-GM which have different growth factor requirements (Kinashi *et al.*, 1989). Similarly, the K-5 clone was obtained by culturing induced LyD9 cells in the media containing IL-5 as a sole growth factor. The existence of the neomycin-resistant gene which had been introduced into the parental LyD9 clone as a genetic marker verified that the K-5 clone originated from LyD9 cells (data not shown).

Figure 1 shows FACS analysis of the surface phenotypes expressed on K-5 cells: $B220^+$, IgM^-, $Thy1.2^-$, $CD3^-$, $Ia^\pm$, $Fc\gamma R^+$, $Mac\text{-}1^+$ and $F4/80^+$. The surface phenotypes of K-5 cells were similar to those of LyD9 cells (Kinashi *et al.*, 1988, 1989), except that K-5 cells expressed less B220 and slightly more Mac-1 antigens. K-5 cells had lymphoid characteristics: mononuclear cells with round nuclei, medium to large nuclear:cytoplasmic ratios and negligible amounts of α-naphthylbutyrate esterase and naphthol AS-D chloroacetate esterase (Figure 2A and C). Myeloperoxidase staining was negative (data not shown).

The growth factor requirements of LyD9 and K-5 cells are compared in Table I. LyD9 cells were strictly IL-3 dependent whereas K-5 cells responded to IL-5 as well as IL-3. A weak response to IL-4 was seen in K-5 as well as LyD9 cells. K-5 cells could grow in the presence of IL-5 or IL-3 for a long time. However, IL-4 could not support long-term proliferation of K-5 as well as LyD9 cells. K-5 cells did not respond to IL-6, IL-7, GM-CSF, G-CSF or M-CSF.

When K-5 cells were co-cultured with the PA6 stroma cell line in the presence of IL-5, K-5 cells began to grow in direct contact with PA6 cells. After 4 weeks the cells were harvested for cytochemical characterization. Unlike the original K-5 cells, most of the co-cultured cells had a large cytoplasm, and some (~5% of the cells) had a band-like or segmented nucleus, which is characteristic of granulocytes (Figure 2B). About 50% of the harvested cells seemed to be granulocytes as they were positive for naphthol AS-D chloroacetate esterase (Figure 2D). About 10% of the co-cultured cells seemed to be macrophages as they were strongly positive for α-naphthylbutyrate esterase (Figure 2D).

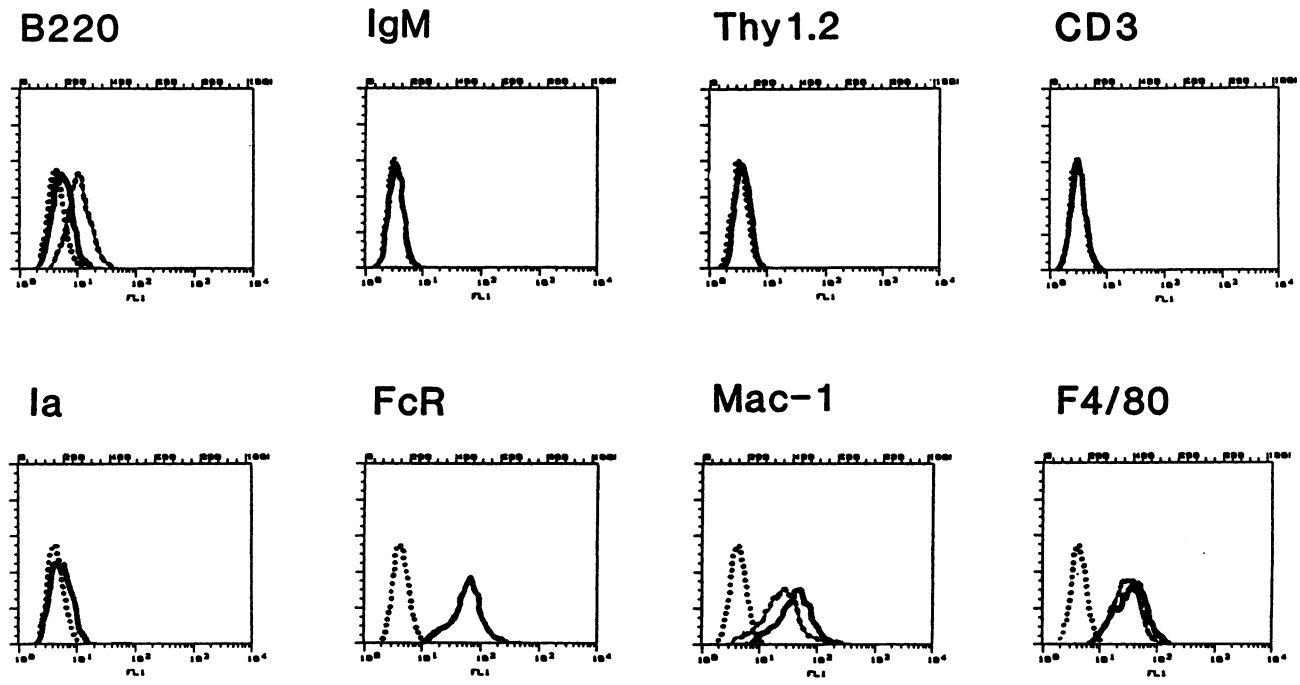

Fig. 1. Surface phenotypes expressed on K-5 cells. Broken lines represent negative controls in which K-5 cells were stained without the first antibody. Thick lines represent expression of cell surface antigens detected by the antibodies indicated. Thin lines in B220, Mac-1 and F4/80 antigens represent their expression profiles on LyD9 cells.

LT-5, transformant of K-5

Co-culture of K-5 cells with another stroma cell line, ST-2s10 (ST2) resulted in overgrowth of transformant cells before we could identify myeloid lineage cells. Eight transformant cell lines derived from K-5 cells were thus obtained which grew without any exogenous growth factors. One of them seemed to produce IL-3 because its supernatant supported the growth of LyD9 cells (data not shown). Another six transformant cell lines showed no evidence for secreting any growth factors that can support the growth of LyD9, K-4, K-5 and LS-1 cells. The remaining transformant, LT-5, was extensively analyzed in this study. Surface phenotypes of LT-5 cells were similar to those of K-5 cells: $B220^+$, IgM^-, $CD3^-$, $Ia^\pm$, $Fc\gamma R^+$, Mac-1^+, $F4/80^+$. Cytochemical staining of LT-5 cells revealed the absence of myeloperoxidase, naphthol AS-D chloroacetate esterase and α-naphthylbutyrate esterase.

Tumorigenicity of LT-5 cells was tested in nude mice. All of five adult nude mice (BALB/c female) that had been inoculated s.c. with LT-5 cells (1.0×10^7 cells) bore tumors in 2 weeks. The inoculated nude mice developed generalized lymph node swelling and hepatosplenomegaly in 4 weeks and died in 6 weeks. Figure 3 shows the histology of the affected lymph node and spleen. The lymph node was swollen, devoid of normal follicles and diffusely infiltrated by LT-5 cells. The spleen was infiltrated by LT-5 cells mostly in the white pulp (Figure 3C). The peripheral blood of the mice bearing LT-5 tumors did not show apparent eosinophilia (data not shown).

Autocrine growth of LT-5 cells by secretion of IL-5

To understand molecular mechanisms of transformation of LT-5 cells, we examined whether LT-5 conditioned medium (CM) contained any growth factor activities using several factor-dependent cell lines: FDC-P2, LyD9, K-4, K-5 and LS-1 (Figure 4). Among these, only K-5 cells that respond to IL-3 or IL-5 proliferated in the presence of LT-5 CM. Neither IL-3-dependent cell lines such as FDC-P2 and LyD9 cells nor IL-4-dependent K-4 cells responded to LT-5 CM. Furthermore, LS-1 cells, which could clearly respond to IL-3, IL-4, GM-CSF, G-CSF and M-CSF, hardly proliferated with LT-5 CM. These results suggest that LT-5 CM contains the IL-5 activity. The IL-5 activity of LT-5 CM was confirmed by the observation that the addition of the anti-IL-5 antibody (NC-17) clearly inhibited the growth

Table I. Responses of LyD9 and K-5 to growth factors

Growth factors added	[^{3}H]Thymidine incorporation (c.p.m.)	
	LyD9	K-5
Medium	360	380
hIL-1α, 100 U/ml	420	670
hIL-2, 100 U/ml	660	740
mIL-3[a]	26 500	18 000
mIL-4[b]	1 450	2 600
mIL-5[c]		
0.1%	ND	18 500
1%	ND	49 300
10%	320	65 300
hIL-6, 50 U/ml	220	460
mIL-7, 25 U/ml	ND	390
mGM-CSF, 10 U/ml	200	550
hG-CSF[d]	150	850
mM-CSF[e]	210	470

LyD9 and K-5 cells (3×10^5/ml) were incubated in a culture medium containing growth factors indicated for 48 h. h, human; m, murine. Data are means of triplicate experiments. ND, not done.
[a]10% WEHI-3B conditioned medium.
[b]10% conditioned medium of X63Ag8 producing IL-4.
[c]0.1 – 10% conditioned medium of X63Ag8 producing IL5.
[d]0.1% partially purified human G-CSF solution.
[e]20% L cell conditioned medium.

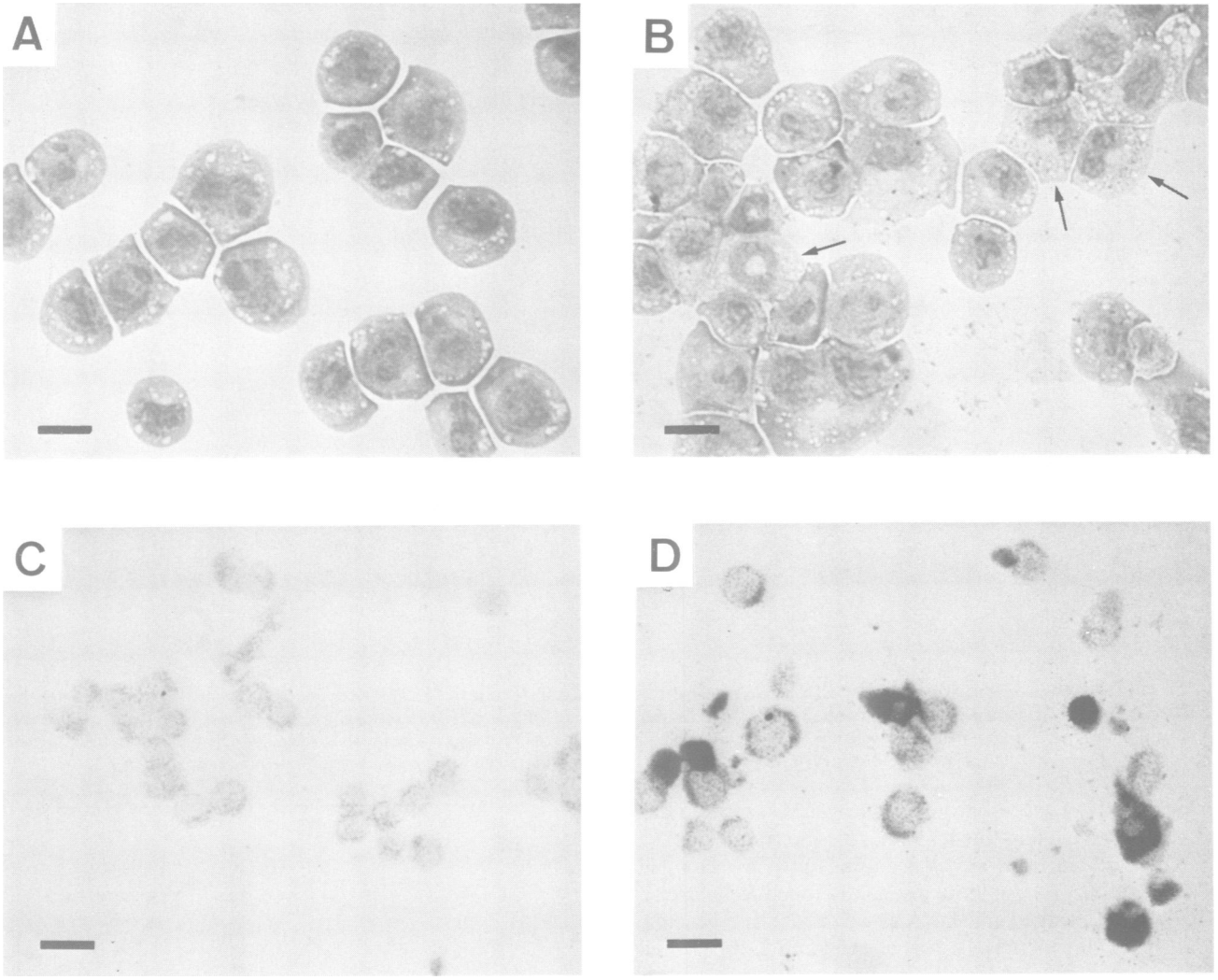

Fig. 2. The morphology of K-5 cells (**A** and **C**) and differentiation-induced K-5 cells by co-culture with PA6 cells (**B** and **D**). (**A**, **B**) May–Grünwald–Giemsa stain, 312× (bars are 10 μm). (**C**, **D**) Esterase double stain, 156× (bars are 20 μm). In (B), arrows show mature granulocytes. In (C) and (D), blue and brown granules represent naphthol AS-D chloroacetate esterase and α-naphthylbutyrate esterase in esterase stain respectively.

of K-5 cells cultured in the presence of LT-5 CM as shown in Figure 5. The results suggest that the spontaneous growth of LT-5 cells may be ascribed to the autocrine proliferation by secretion of IL-5.

The growth of LT-5 *per se* was also suppressed by the addition of anti-IL-5 antibody in a dose-dependent fashion when added on day 0 as shown in Figure 6. Although the growth inhibition of LT-5 by the anti-IL-5 antibody was transient, daily addition of the anti-IL-5 antibody strongly inhibited the growth of the LT-5 cells. The growth of LT-5 cells was less affected by the anti-IL-5 antibody than that of K-5 cells (data not shown). On the other hand, the addition of the anti-IL-4 monoclonal antibody (11B11) that belongs to the same subclass as the anti-IL-5 antibody did not affect the growth of the LT-5 cell at all (data not shown). Needless to say, the same concentrations of the anti-IL-5 antibody had no suppressive effects on the growth of other cell lines such as LyD9, K-4, and LS-1, which do not require IL-5 for growth. These results support the idea that LT-5 cells proliferate in an autocrine manner by secretion of IL-5 and that secreted IL-5 serves as the exogenous growth factor of LT-5 cells.

Transposition of an IAP element activates the IL-5 gene in LT-5 cells

We confirmed the production of IL-5 in LT-5 cells by the presence of IL-5 mRNA. Poly(A)$^+$ RNAs were isolated from LT-5 cells and analyzed by Northern blot hybridization with the murine IL-5 cDNA probe (Figure 7). LT-5 cells contained 2.2 kb IL-5 mRNA, which is larger than normal IL-5 mRNA (1.7 kb) of C3H spleen cells. Parental K-5 cells did not contain any detectable amount of IL-5 mRNA, as expected.

Expression of the IL-5 mRNA of the aberrant size suggests that DNA rearrangement might have taken place in the vicinity of the IL-5 gene of LT-5 cells. In fact, Southern blot hybridization of LT-5 DNA with the IL-5 cDNA probe showed that LT-5 cells contained an extra 4.7 kb *Eco*RI band in addition to the 5′ (4 kb) and 3′ (9.6 kb) *Eco*RI fragments of the IL-5 gene (Figure 8B). The 5′ genomic DNA probe of the IL-5 gene hybridized with the extra 4.7 kb IL-5 gene fragment of LT-5 cells, indicating that DNA rearrangement occurred at the 5′ flanking region of one allele of the IL-5 gene in LT-5 cells.

Since the integration of the IAP genome has been reported

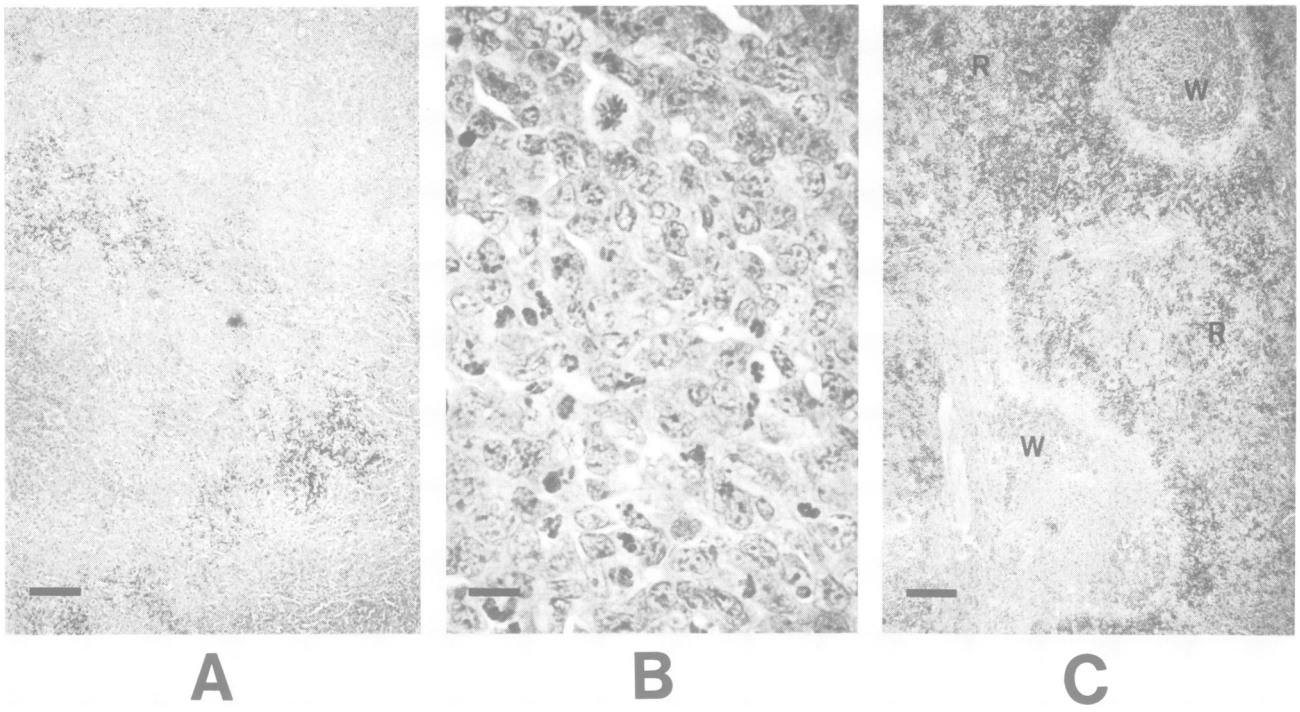

Fig. 3. Histology of the affected lymph node and the spleen of the LT-5-injected nude mouse. (**A**) Hematoxylin–eosin-stained lymph node, 40× (bar is 200 μm). Normal lymphoid follicles disappeared and LT-5 cells were diffusely infiltrated. (**B**) Hematoxylin–eosin-stained lymph node, 400× (bar is 20 μm). There are clusters of large and highly atypical cells with some inflammatory cells scattered among them. (**C**) Hematoxylin–eosin-stained, spleen, 27× (bar is 200 μm). The white pulps (W) are invaded by large LT-5 cells. Some LT-5 cells also infiltrated the red pulp (R).

in the vicinity of several growth factor genes, we tested this possibility by cloning the 4.7 kb IL-5 gene fragment. The 4.7 kb *Eco*RI fragment was cloned using λgt*WES* vector and designated λIL-5-4.7 and its insert was found to hybridize with the IAP total genomic DNA as well as the IL-5 genomic DNA. The λIL-5-4.7 DNA was cleaved into 2.25, 1.7 and 0.75 kb fragments by *Hin*dIII digestion. Among these, the 5′ exon of the IL-5 gene (probe b in Figure 8A) hybridized with the 2.25 kb band in addition to the 3.0 kb partial digest (Figure 9A, lane 1). The *Hin*dIII fragments hybridized with the IAP total genomic DNA (probe c in Figure 10) were the 1.7 and 0.75 kb bands in addition to the 3.0 and 2.45 kb partial digests (Figure 9A, lane 2). The 3.0 kb partial digest hybridized with both probes. Only one (2.9 kb) of the two *Sac*I fragments hybridized with both probes b and c (Figure 9A, lanes 3 and 4). The *Xba*I site located 5′ to the coding region in the IL-5 gene was missing in λIL-5-4.7. Taken together, the above results allowed us to locate the IAP element at the 5′ flanking region of the rearranged IL-5 gene in LT-5 cells (Figure 10). The 5′ portion (probe d) of the IAP-LTR (R and U5 regions) hybridized with the 0.6 kb *Sac*I–*Pst*I fragment (Figure 9B, lane 1). The U3 sequence (probe e) of the IAP-LTR hybridized with the 1.85 kb *Sac*I–*Pst*I fragment (Figure 9B, lane 2). The results summarized in Figure 10 showed that the IAP-LTR was located immediately 5′ to the IL-5 gene in the same orientation as the transcription of the IL-5 gene. The IAP internal sequence is located further upstream of the LTR.

To confirm the transposition of the IAP-LTR in the 5′ flanking region of the IL-5 gene, we determined the nucleotide sequence of λIL-5-4.7 DNA using oligonucleotide primers. We found the R and U5 region sequences of the IAP-LTR, which were 96 and 95% homologous to those of MIA14 and rc-*mos* respectively (Christy *et al.*, 1985),

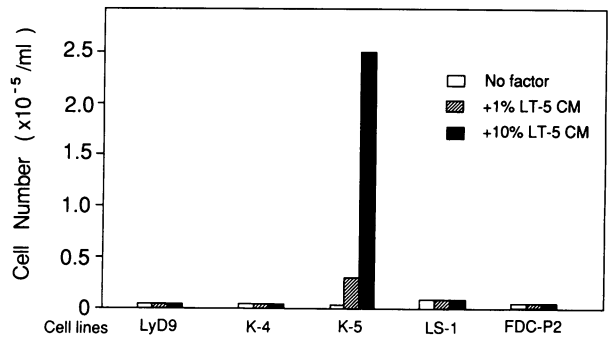

Fig. 4. Effects of LT-5 CM on growth of several factor-dependent cell lines. Each cell line (1.0×10^5 cells/ml) was incubated with or without LT-5CM and viable cells were counted after 72 h culture.

upstream of position 194 of the IL-5 gene (Figure 11). The downstream IL-5 gene sequence was identical to the germ line IL-5 gene sequence (Mizuta *et al.*, 1988) except for one additional A base at position 141, which could be explained by a cloning artefact or polymorphism. The location of the IAP-LTR suggests that the promoter of the IL-5 gene was replaced by the LTR promoter, which is located ~330 bp upstream of that of the germ line IL-5 gene, resulting in the constitutive expression of IL-5 mRNA of the aberrant size roughly as expected by Northern blot hybridization.

Discussion

We have established a murine myeloid progenitor cell line, K-5, that requires either IL-5 or IL-3 for its growth. This cell line was derived from the IL-3-dependent hemopoietic progenitor cell line LyD9 by co-culture with bone marrow stroma cells, followed by selective growth in the presence

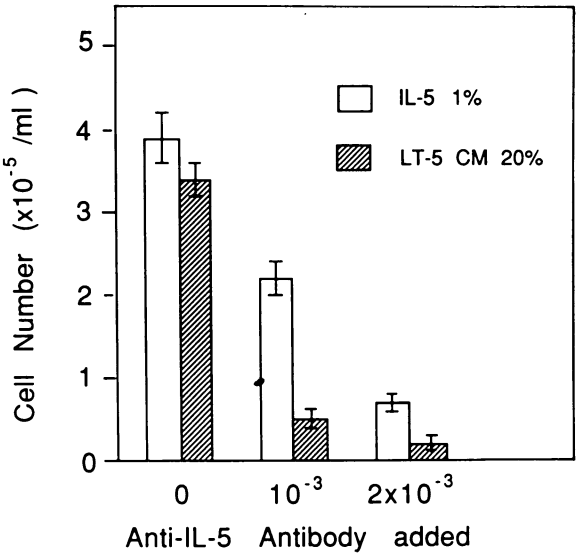

Fig. 5. Effects of anti-IL-5 monoclonal antibody on the IL-5 activity of LT-5CM. K-5 cells (1.0×10^5 cells/ml) were incubated with 1% CM of X63Ag8 producing IL-5 or 20% LT-5 CM in the absence or presence of anti-IL-5 (the final concentration was ×1000 or ×500 dilution of the original ascites). Viable cells were counted after 48 h culture. Each column shows the data of triplicate experiments with standard deviation.

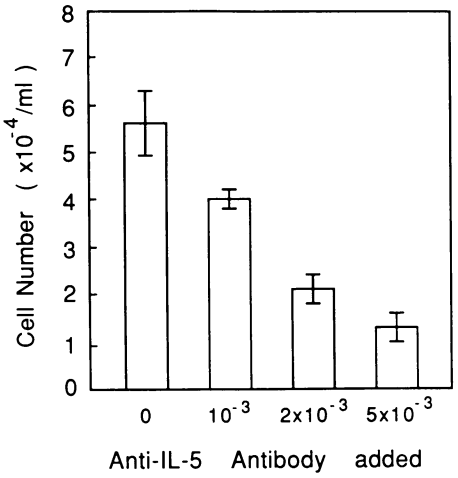

Fig. 6. Effect of anti-IL-5 monoclonal antibody on growth of LT-5 cells. LT-5 cells (1×10^4/ml) were incubated in the absence or presence of anti-IL-5 (the final concentration was ×1000, ×500 or ×200 dilution of the original ascites). Viable cells were counted after 48 h culture. Each column shows the data of triplicate experiments with standard deviation.

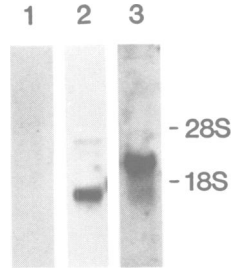

Fig. 7. Expression of IL-5 mRNA in LT-5 cells. **Lane 1**, 8 μg of poly(A)$^+$ RNA of K-5 cells; **lane 2**, 2 μg of poly(A)$^+$ RNA of phytohemagglutinin (PHA)-stimulated spleen cells from C3H mice; **lane 3**, 8 μg of poly(A)$^+$ RNA of LT-5 cells. Probe used is the *Bam*HI−*Acc*I fragment of the murine IL-5 cDNA clone (probe a in Figure 8A).

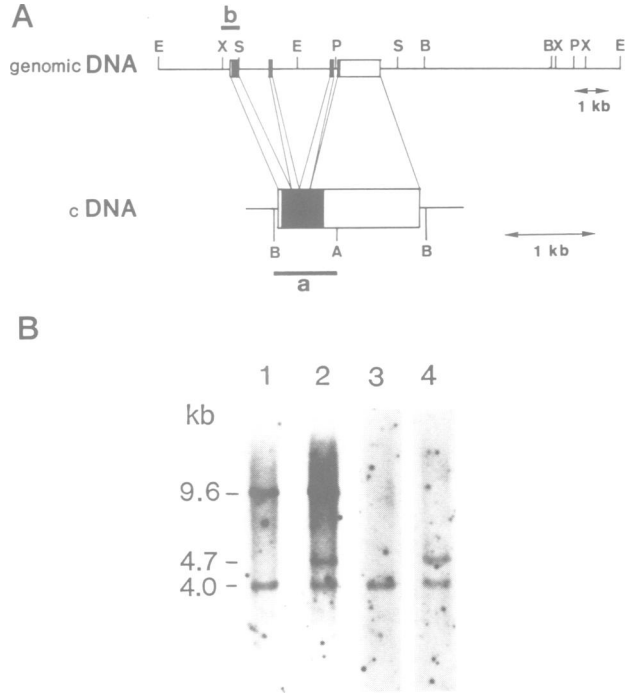

Fig. 8. Rearrangement of the IL-5 gene in LT-5 cells. (**A**) Restriction site map of the murine IL-5 genomic DNA and cDNA. Closed and open boxes indicate coding and untranslated regions respectively. Vertical lines indicate restriction sites: E, *Eco*RI; X, *Xba*I; P, *Pst*I; S, *Sac*I; B, *Bam*HI; A, *Acc*I. Bars (a) and (b) show IL-5 cDNA and genomic DNA probes used in this study, respectively. (**B**) Southern blot hybridization analysis. *Eco*RI-digested DNAs (6 μg) of K-5 cells (**lanes 1** and **3**) and LT-5 cells (**lanes 2** and **4**) were electrophoresed and blotted. Probes used were the *Bam*HI−*Acc*I fragment of murine IL-5 cDNA clone (probe a) for **lanes 1** and **2**, and the *Xba*I−*Sac*I fragment of murine IL-5 genomic DNA (probe b) for **lanes 3** and **4**.

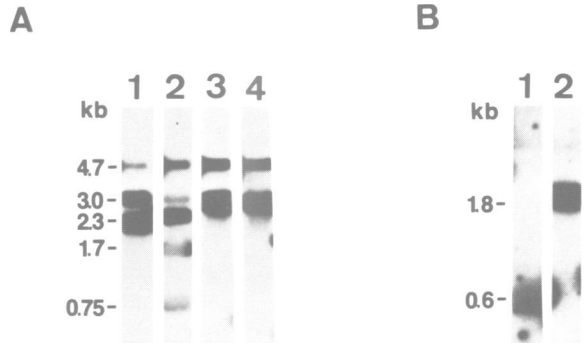

Fig. 9. Structural characterization of the cloned IL-5 gene of LT-5 cells by Southern blot hybridization analysis. The insert of λIL-5-4.7 was isolated and digested with the restriction enzymes described below. (**A**) **Lanes 1** and **2**, *Hin*dIII; **lanes 3** and **4**, *Sac*I. (**B**) **Lanes 1** and **2**, *Sac*I and *Pst*I. Probes used were (**A**) **lanes 1** and **3**, the *Xba*I−*Sca*I fragment of murine IL-5 genomic DNA (probe b in Figure 8A); **lanes 2** and **4**, the 6.8 kb fragment of the IAP total genome, 81A(+) (probe c in Figure 10); (**B**) **lane 1**, 5′ portion of the IAP-LTR from 81C(+) (probe d in Figure 10); **lane 2**, the U3 sequence of the IAP-LTR from 81C(+) (probe e in Figure 10).

of IL-5. The precursor−product relationship is verified by the presence of the neomycin resistance gene in K-5 with the same integration profile as with the parental LyD9. K-5 cells co-cultured with PA6 cells clearly differentiated into the myeloid lineage cells, granulocytes and macrophages in

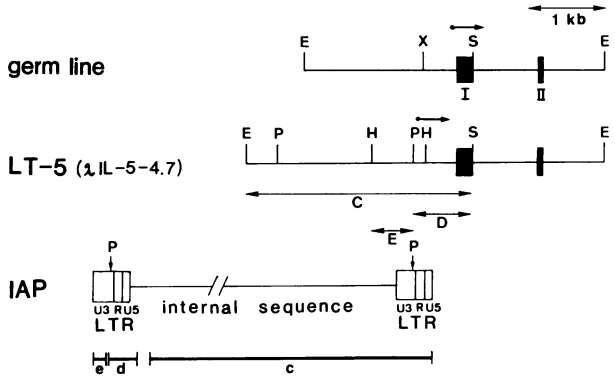

Fig. 10. Restriction site map of the 5′ flanking region of the IL-5 gene in the germ line and LT-5 cells. Closed boxes (I and II) indicate the coding regions. Vertical lines indicate restriction sites: E, *Eco*RI; X, *Xba*I; P, *Pst*I; H, *Hin*dIII; S, *Sac*I (see also Figure 8A). The structure of an IAP element and the positions of the probes are shown below. (c) The 6.8 kb fragment of the IAP total genome from 81A(+); (d) 5′ subdivision of the IAP-LTR from 81C(+); (e) U3 sequence of the IAP-LTR from 81C(+). (d) and (e) are divided by the *Pst*I site. (C), (D) and (E) indicate the regions hybridized with probes (c), (d) and (e) respectively. The arrows show the initiation sites and the direction of transcription.

agreement with the myelopoietic capacity of PA6 (Kodama *et al.*, 1984). IL-5 is considered to support the growth and differentiation of early B cells (Takatsu *et al.*, 1980; Kinashi *et al.*, 1986), immature T cells (Takatsu *et al.*, 1987) and precursors of eosinophils (Yokota *et al.*, 1987; Yamaguchi *et al.*, 1988). K-5 cells proliferate by the addition of IL-5 as well as IL-3, but we have been so far unsuccessful in inducing K-5 cells into mature B cells by co-culturing with bone marrow stroma cells, unlike the case of K-4 cells (Kinashi *et al.*, 1989).

The establishment of various progenitor cell lines such as LyD9, K-4 and K-5, which require different growth factors, suggests that different growth factor dependency might correlate with differentiation steps. During bone marrow induction of LyD9, the receptor for IL-5 might have been increased in number or activated by changing its affinity (Mita *et al.*, 1988). Indeed, high-affinity IL-5 receptors were detected on K-5 cells but not on LyD9 cells by receptor-binding assays (unpublished data). In some cases differentiation may induce activation of a signal transduction pathway specific to a growth factor. Further isolation of LyD9 derivatives requiring different growth factors and examination of their differentiation capacities will allow us to

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LT-5        AATAAAGCTTTGCCGCAGAAGATTCTGGTCTGTGGTGTTCTTCC -224
            ..........................................
rc-mos      AATAAAGCTTTGCCGCAGAAGATTCTGGTCTGTGGTGTTCTTCC
                HindIII      R  U5

IL-5        TTCTTTTATGTTATAGAAAATGCTTTTTAAGCAGGGGTGGGGGT
            .     .. . .    .   .  .............
LT-5        TGGCCGGTCGTGAGAACGCGTCTAATAACAGCAGGGGTGGGGGT -180
            ............................
rc-mos      TGGCCGGTCGTGAGAACGCGTCTAATAACA
                                        U5

IL-5        CAAGATGTTAACTATTATTAAAGAGCAAAAAAAAAAAA-TGCAT
            ..................................... .....
LT-5        CAAGATGTTAACTATTATTAAAGAGCAAAAAAAAAAAAATGCAT -136

IL-5        TTTGTTTGAAGACCCAGGGCACTGGAAACCCTGAGTTTCAGGAC
            ..........................................
LT-5        TTTGTTTGAAGACCCAGGGCACTGGAAACCCTGAGTTTCAGGAC  -92

IL-5        TCGCCTTTATTAGGTGTCCTCTATCTGATTGTTAGCAATTATTC
            ..................................|.....|.....
LT-5        TCGCCTTTATTAGGTGTCCTCTATCTGATTGTTAGCAATTATTC  -48

IL-5        ATTTCCTCAGAGAGAGAATAAATTGCTTGGGGATTCGGCCCTGC
            ..............|.....|....................
LT-5        ATTTCCTCAGAGAGAGAATAAATTGCTTGGGGATTCGGCCCTGC   -4

                  *
IL-5        TCTG
            ....
LT-5        TCTG
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Fig. 11. Comparison of the nucleotide sequences of the 5′ flanking region of the germ line and LT-5 IL-5 genes, and the IAP-LTR. The IL-5 gene sequence is taken from Mizuta *et al.* (1988). Sequences of the R and U5 regions of IAP-LTR are taken from rc-*mos* (Christy *et al.*, 1985). The same nucleotides are indicated by dots. ---, deletion. CAAT-like and TATA-like sequences are boxed. Numbers indicate nucleotide positions from the transcription initiation site of the IL-5 gene (*). The germ line IL-5 gene sequence presented here includes two corrections: deletion of GT at position −91/−90 and insertion of G at position −10.

determine possible pathways of differentiation from LyD9 cells to myeloid lineage as well as B lymphocytes.

Malignant transformation is often caused by aberrant expression of growth factors such as IL-2 (Karasuyama *et al.*, 1989), IL-3 (Ymer *et al.*, 1985; Stocking *et al.*, 1988), IL-4 (Brown *et al.*, 1987), GM-CSF (Lang *et al.*, 1985; Stocking *et al.*, 1988), transforming growth factor (DeLarco *et al.*, 1978; Kaplan *et al.*, 1982) and platelet-derived growth factor (Doolittle *et al.*, 1983; Waterfield *et al.*, 1983). To our knowledge, the LT-5 cell line is the first case that proliferates by autocrine secretion of IL-5. In addition, we have clearly demonstrated that secreted IL-5 affects the growth of LT-5 cells as the exogenous anti-IL-5 antibody inhibited proliferation of LT-5 cells. Apparently random transposition of IAP elements within the murine genome occasionally triggered oncogenesis (Kuff and Lueders, 1988). The activation of c-*mos* oncogene in a mouse plasmacytoma cell line was found to result from the insertion of the IAP element (Canaani *et al.*, 1983). Constitutive synthesis of IL-3 by the WEHI-3B cell line was reported to be due to the insertion of the IAP element (Ymer *et al.*, 1985). We have shown that the IL-5 gene of LT-5 is rearranged by transposition of IAP-LTR. This LTR transposition is likely to be responsible for the aberrant expression of the IL-5 gene because (i) the orientation and location of IAP-LTR allow the constitutive expression of the IL-5 gene and (ii) the extra length ($\sim$0.5 kb) of LT-5 mRNA is almost equivalent to the distance of the promoter in LTR and the endogenous promoter (Figure 11). Although IL-5 mRNA of LT-5 cells is longer, the IL-5-like activity produced by LT-5 cells was effective on IL-5-dependent K-5 cells (Figure 4), and markedly inhibited by anti-IL-5 monoclonal antibody (Figure 5). Therefore, at least the active site of the IL-5 protein produced by LT-5 cells should be kept intact by this transposition.

We have shown that the parental LyD9 cell can be induced to differentiate into myeloid cells without transformation when co-cultured with ST2 cells (our unpublished observation). The different susceptibility to transformation on ST2 cells between LyD9 and K-5 cells might be related to their stages of differentiation, which would affect the chromatin structure of IAP elements and the IL-5 gene, among others. Characterization of other spontaneous transformants of K-5 cells may reveal clues to correlate differentiation stages of progenitor cells and mode of transformation.

Materials and methods

Cell lines and animals

An IL-3-dependent cell line, LyD9, was established from CBA/j mouse bone marrow cells by Dr Palacios (Palacios *et al.*, 1987). LyD9 cells were maintained in RPMI 1640 medium supplemented with 10% FCS, 5% WEHI-3B conditioned medium containing IL-3, 50 μM 2-mercaptoethanol and 2 mM L-glutamine. LyD9 cells used for differentiation induction in this study had been transfected with pSV2-Neo plasmid by electroporation and were resistant to G418 (Sigma Chemicals Co., St Louis, MO, USA). An IL-4-dependent derivative (K-4) and a CSF-dependent derivative (LS-1) of LyD9 have been described previously (Kinashi *et al.*, 1989). FDC-P2 (Dexter *et al.*, 1980) is a strictly IL-3-dependent cell line. Stroma cell clones, PA6 (Kodama *et al.*, 1982) and ST2s10 derived from ST2 (Ogawa *et al.*, 1988) were generously provided by S.Nishikawa (Kumamoto University, Japan). The stroma clones were cultured in RPMI 1640 medium with 10% FCS, 50 μM 2-mercaptoethanol and 2 mM L-glutamine. Cell morphology was examined by May–Grünwald–Giemsa stain, and cytochemical staining was done using myeloperoxidase, naphthol AS-D chloroacetate esterase and α-naphthylbutyrate esterase staining kits (Mutoh Chemicals, Japan). Adult female mice of NZB, C3H and BALB/c nude strains were purchased from Shizuoka Laboratory Animal Center (Shizuoka, Japan).

Differentiation induction by co-culturing with stroma cells

Differentiation induction of LyD9 cells with bone marrow stroma cells was done as described previously (Kinashi *et al.*, 1988). Briefly, bone marrow stroma cells were prepared by culturing bone marrow cells from NZB mice for 5 days. After removing non-adherent cells, LyD9 cells were layered on the stroma cells in RPMI 1640 medium containing 10% FCS, 50 μg/ml lipopolysaccharide (Difco Laboratories, Detroit, MI, USA) and 50 μg/ml agar extract mitogen. Non-adherent cells were harvested on day 10 to obtain induced LyD9 cells. K-5 cells were established by cultivating induced LyD9 cells in the medium containing IL-5 [10% conditioned medium of X63Ag8 myeloma cells transfected with murine IL-5 cDNA (Kinashi *et al.*, 1986)] and 1 mg/ml G418 without IL-3. Co-culture of K-5 and stroma cell lines (PA6 or ST2) was done in the medium used for the stroma cells containing 5% IL-5 for 4 weeks.

Antibodies

The antibodies used in this study were as follows: fluorescein isothiocyanate (FITC)-labeled goat anti-mouse IgM (Cooper Biomedical, West Chester, PA, USA), FITC-labeled anti-Thy1.2 (Miles Scientific, Naperville, Israel), anti-Ia^k (Meiji Institute of Health Science, Japan), 6B2 as anti-B220 (Coffman, 1983), 2C11 as anti-CD3 (Leo *et al.*, 1987), 2.4 G2 as anti-Fcγ receptor (FcR) (Unkeless, 1979), M1/70 as anti-Mac-1 (Springer *et al.*, 1979) and F4/80 (Austin and Gordon, 1981). Unlabeled antibodies were facilitated with FITC-labeled goat anti-rat IgG antibody. For anti-Ia^k, FITC-labeled goat anti-mouse IgG was used as the second antibody. 2C11 was biotinylated and FITC–avidin was used for staining. Stained cells were analyzed by FACScan (Becton-Dickinson & Co., Mountain View, CA, USA). Anti-murine IL-5 monoclonal antibody (NC-17) (Harada *et al.*, 1987) was generously provided by K.Takatsu (Kumamoto University, Japan). Anti-murine IL-4 monoclonal antibody (11B11) (Ohara and Paul, 1985) was kindly provided by W.Paul (NIH, Bethesda, MD, USA).

Cell proliferation assays

Aliquots of cell suspensions were incubated in a culture medium containing growth factors in a 96-well microplate for 48 or 72 h. Growth response was measured by [^{3}H]thymidine incorporation (0.5 μCi/well; Amersham, Japan) during the last 6 h of culture, or by counting viable cells using a dye (trypan blue) exclusion assay. The growth factors used were: human recombinant (r) IL-1α (Dainippon Pharmaceutical Co., Japan), human rIL-2 (Takeda Pharmaceutical Co., Japan), WEHI-3B conditioned medium for murine IL-3, conditioned medium of X63Ag8 myeloma cells transfected with murine IL-4 and IL-5 cDNAs (Kinashi *et al.*, 1986; Noma *et al.*, 1986; Karasuyama and Melchers, 1988) for IL-4 and IL-5 respectively, human rIL-6 (Hirano *et al.*, 1986), conditioned medium of COS-7 cells transfected with murine IL-7 cDNA (kindly provided by T.Sudo of the Biomaterial Research Institute, Japan) for IL-7 (Namen *et al.*, 1988), murine rGM-CSF (Kajigaya *et al.*, 1986), partially purified human rG-CSF (Nagata *et al.*, 1986), and L cell conditioned medium for murine M-CSF (Waheed and Shadduck, 1979).

Isolation and characterization of nucleic acids

High mol. wt DNA was isolated as described previously (Yaoita and Honjo, 1980). Cloning of a rearranged IL-5 gene was carried out using λgt*WES* DNA as a vector and the *Xba*I–*Sac*I fragment containing the 5′ region of the murine IL-5 gene as a probe (Mizuta *et al.*, 1988). Total RNA was isolated by using guanidine thiocyanate–cesium chloride (Fluka Chemie, Bucks, Switzerland) and poly(A)$^+$ RNA was purified by using an oligo(dT)–cellulose column (Pharmacia, Uppsala, Sweden). Northern blot hybridization (Makmaster and Carmichael, 1977) and Southern blot hybridization (Southern, 1975; Yaoita and Honjo, 1980) were performed as described using probe DNA fragments labeled with [α-^{32}P]dCTP (Amersham, Japan), and hybridized filters were autoradiographed. Probes used were: the *Bam*HI–*Acc*I fragment of murine IL-5 cDNA clone (Kinashi *et al.*, 1986); the *Xba*I–*Sac*I fragment of the murine IL-5 genomic DNA (Mizuta *et al.*, 1988). The 6.8 kb *Eco*RI fragment [81A(+)] and the 3.3 kb *Eco*RI fragment [81C(+)] of the IAP element were used for the IAP total genome and the IAP-LTR respectively (Cole *et al.*, 1981). Both IAP DNAs were generously provided by M.Ono (Kitazato University, Japan). Nucleotide sequences were determined by the dideoxy chain termination method (Sanger

et al., 1977) using oligonucleotides in the 5′ flanking region of the IL-5 gene as primers.

Acknowledgements

We are grateful to Ms Junko Kuno, Saeko Okazaki and Yumiko Hoshikawa for their technical assistance, and Ms Kazuko Hirano for her help in preparing the manuscript. This investigation was supported by grants from the Ministry of Education, Science and Culture and from the Tokyo Biochemical Research Foundation.

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Received on January 15, 1990; revised on March 20, 1990