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Expression of receptor for ecotropic murine leukemia virus on hematopoietic cells

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Summary. A fusion protein (F-SU/GFP) which is comprised of the surface (SU) subunit of the Friend MuLV envelope glycoprotein and the green fluorescence protein (GFP) was generated by a baculovirus expression system. The F-SU/GFP specifically bound to mammalian tissue cultured cells expressing the mCAT-1, the receptor for ecotropic murine leukemia virus (Eco-MuLV). The expression level of mCAT-1 on hematopoietic cells was measured based on the capacity of cells to absorb the F-SU/GFP. In BALB/c mice susceptible to Eco-MuLV infection, all hematopoietic cell subpopulations absorbed the F-SU/GFP with the most prominent absorption observed in the $TER119^+$ erythroblasts. Hematopoietic cells from C4W and AKR mice did not absorb the F-SU/GFP as readily as the BALB/c cells, probably due to expression of the envelope glycoproteins of endogenous Eco-MuLVs in these mice. Although AKR mice are susceptible to Eco-MuLV infection while C4W mice are resistant, these mice showed no apparent difference in the F-SU/GFP-absorbing capacity.

Introduction

Murine leukemia viruses (MuLVs) have been classified into several subgroups mainly based on their receptor usage. The receptor for ecotropic MuLV (Eco-MuLV) is a cationic amino acid transporter, mCAT-1 [1], whose mRNA is expressed in various organs [12]. The physiological importance of the *mCAT-1* gene was demonstrated by abnormalities in *mCAT-1* gene knockout mice [23]. The *mCAT-1* knockout mice were smaller in size at birth than normal mice and

died immediately after birth with severe anemia. In this study, we prepared a soluble surface (SU) protein, an mCAT-1 binding protein, of ecotropic Friend MuLV by a baculovirus expression system to determine the expression level of mCAT-1 on the surface of mouse hematopoietic cells. In the previous studies, MuLV SU proteins expressed by the baculovirus system were successfully used for the study of receptor binding [3, 4]. The soluble SU we prepared was tagged with a green fluorescent protein (GFP) to be specifically traced.

Cultured cells differ in their SU-absorbing capacity depending on the status of SU expression of Eco-MuLV [9]. The SU expressed by persistently infected cells or those carrying endogenous viral genomes is supposed to interact with the receptors, to inhibit binding of exogenous viral SU to the cells, and thereby to prevent superinfection. We examined three mouse strains; BALB/c mice are susceptible to Eco-MuLV infection and scarcely express SU of endogenous Eco-MuLV, and C4W and AKR mice express the SU of endogenous Eco-MuLV. C4W mice are an *Fv-4*^{r} congenic strain on a BALB/c genetic background [22]. The *Fv-4*^{r} gene is a truncated endogenous MuLV locus that expresses the Eco-MuLV envelope (Env) glycoprotein and confers resistance to infection by exogenous Eco-MuLV [6, 8]. C4W lymphocytes did not absorb the Friend MuLV virions [14]. AKR mice have a few endogenous Eco-MuLV loci, express infectious Eco-MuLV throughout their life, and spontaneously develop T-cell lymphoma, whereas AKR mice are susceptible to exogenous Eco-MuLV infections [25].

Materials and methods

Mice and cell lines

BALB/cAJcl mice were purchased from Clea Japan, Inc., Japan. BALB/c-*Fv-4*^{r} (C4W) mice are a partial congenic mouse strain carrying the *Fv-4*^{r} gene on a BALB/c genetic background [22] and were maintained in our laboratory. AKR/NSlc mice were purchased from Nihon SLC, Hamamatsu, Japan. Five to ten-week-old mice were analyzed.

NIH3T3 cells are of *Mus musculus* origin. SIRC cells were derived from rabbit, and SIRC-NIH EcoR cells were established by transfection of the SIRC cells with the *mCAT-1* gene [27]. FL21 cells are NIH3T3 cells transfected with a DNA construct combining the *Fv-4*^{r} MuLV region and its putative promoter region [18]. Sf21AE and Tn5 B1-4 insect cell lines were derived from *Spodoptera Frugipeda* or *Trichoplusia ni*.

Construction of the F-SU/GFP

A portion of Friend MuLV clone 57 [16] (from nucleotide position (nt) 5636 to 8328) including the entire Env coding region was cloned into the pBluescript II phagemid vector (Stratagene, La Jolla, CA). A 2.2-kb fragment generated by digestion at the *Sfc*I restriction sites of the pBluescript II vector and the Friend MuLV Env (nt 7179) was blunt-ended and inserted into the *Sma*I restriction site of the pEGFP-1 vector (Clontech Laboratories, Palo Alto, CA) to join it to the *GFP* gene in frame. The F-SU/GFP fusion gene was amplified by PCR with primers at the N-terminus of the SU (5′-CCACCAGCAGAATCGACAT-3′) and the C-terminus of the GFP (5′-CCGCTTTACTTGTACAGCTC-3′), and the PCR fragment was inserted into the *Bam*HI restriction site of the pAcYM1 baculovirus transfer vector [21]. Prior to the insertion, the termini of the *Bam*HI digested pAcYM1 were blunt-ended and added with thymidine [19].

Expression of protein in insect cells

To generate recombinant viruses, the recombinant transfer vector and a *Bsu*36I-digested baculovirus DNA (AcRP23.LacZ) [24] were co-transfected into Sf21AE cells with lipofectin (Life Technologies, Rockville, MD). After plaque purification, recombinant viruses producing the F-SU/GFP were selected by Western blotting for the lysates of virus infected cells with goat anti-Rauscher MuLV gp70 antibody (provided by the Division of Cancer Cause and Prevention, National Cancer Institute, Bethesda, MD). A recombinant virus clone was infected to Tn5 B1-4 cells at a multiplicity of infection of 2, and the serum-free culture supernatant of the cells was collected 3 days after infection.

The concentration of the F-SU/GFP protein in one lot of the culture supernatant was estimated by the comparison with the recombinant GFP protein (rEGFP) of known concentration (more than 95% purity; Clontech) on SDS-polyacrylamide gel. The supernatant was concentrated by Ultrafree-MC Centrifugal Filter Units (3000 NMWL) (Millipore, Bedford, MA) and fractionated on 9% SDS-PAGE, together with various dilutions of the rEGFP (Clontech). The gel was stained with GelCode Blue Stain Reagent (Pierce, Rockford, IL), and the intensities of the 87 kD F-SU/GFP and the 27 kD rEGFP were calculated by LAS-1000plus (Fujifilm, Tokyo, Japan) and Image Gauge (Fujifilm). Thus, we roughly estimated the concentration of the F-SU/GFP to be 2 μg/ml. The concentration of the F-SU/GFP was also confirmed by comparison of the reactivity to anti-GFP antibody in Western blot assay with that of the rEGFP. This culture supernatant was used as the F-SU/GFP solution throughout the experiments.

Western blotting

Ten μl of culture supernatant of the cells infected with the F-SU/GFP-producing virus or the parental AcRP23.LacZ virus was fractionated on 7.5% SDS-polyacrylamide gel. Proteins were transferred onto a polyvinylidene difluoride membrane (Immobilon; Millipore) and incubated in PBS containing 5% ECL Blocking agent (Amersham Pharmacia, Buckinghamshire, England) and 0.05% Tween-20 to block nonspecific bindings of the antibodies. The membrane was probed with horseradish peroxidase (HRP)-conjugated anti-GFP antibody (Living Colors Peptide Antibody-HRP conjugate; Clontech); then HRP-mediated chemiluminescent reaction was performed with ECL Plus Western Blotting Detection Reagents (Amersham Pharmacia). The same membrane was reprobed with biotin-labeled goat anti-Rauscher MuLV gp70 antibody and HRP-conjugated streptavidin (Amersham Pharmacia) after stripping of the first probed antibody with Restore Western Blot Stripping Buffer (Pierce).

Flow cytometry

Mammalian tissue cultured cells were harvested from dishes with Hanks buffer containing 0.25% trypsin and 1 mM EDTA-4Na (Life Technologies) and washed with ice-cold washing buffer (PBS containing 5% FCS, and 0.05% NaN_3). 4×10^5 cells were incubated in 0.5 ml of PBS containing the 10% insect cell culture supernatant including the F-SU/GFP, 5% FCS, and 0.05% NaN_3 for 1 h at 5 °C with rotation. After washing with the ice-cold washing buffer, cells were immediately fixed with PBS containing 1% paraformaldehyde and analyzed by an EPICS XL flow cytometer (Beckmann-Coulter, Fullerton, CA) and WinMDI software version 2.8.

Single-cell suspensions from the bone marrow (BM), thymus, and spleen of mice were prepared by mincing and pipetting cells in Dulbecco's Modified Eagle medium (D-MEM) (Nissui, Tokyo, Japan) containing 1% FCS and 0.05% NaN_3. To lyze erythrocytes before antibody treatments, spleen cells suspended in D-MEM containing 10% FCS were added with 3 volumes of 0.85% NH_4Cl and incubated for 10 min on ice. The buffer was then re-

placed by PBS containing 5% FCS, and 0.05% NaN_3. 3×10^6 of hematopoietic cells were first incubated with the anti-CD16/32 antibody (Phar Mingen, San Diego, CA) for 30 min on ice to block nonspecific binding of the antibodies to the Fc receptors, then incubated in 40 μl or 150 μl of the F-SU/GFP solution (culture supernatant) for 1 h on ice. After washing with ice-cold washing buffer, the cells were stained with mixtures of the rabbit anti-GFP antibody (MBL, Nagoya, Japan) and specific antibodies against the cell surface markers for 1 h on ice, followed by washing with the ice-cold washing buffer. The following monoclonal antibodies were used to detect the cell surface markers: phycoerythrin (PE)-labeled anti-TER119 (Ly-76) (Phar Mingen); biotin-labeled anti-Mac-1 (CD11b/CD18) (Dainippon Pharmaceutical, Osaka, Japan); PE-labeled anti-CD3 (Phar Mingen); biotin-labeled anti-Thy 1.2 (Becton Dickinson, Franklin Lakes, NJ); and biotin-labeled anti-surface IgM (sIgM) (Zymed, San Francisco, CA). The cells treated with PE-labeled antibodies were then stained with fluorescein isothiocyanate (FITC)-labeled anti-rabbit IgG (Dako, Glostrup, Denmark), and the cells treated with biotin-labeled antibodies were stained with a mixture of FITC-labeled anti-rabbit IgG and PE-labeled streptavidin (Phar Mingen) for 30 min on ice. The cells were fixed with PBS containing 1% paraformaldehyde after the final washing and analyzed by an EPICS XL flow cytometer and WinMDI software. A total of 3×10^4 cells per sample was analyzed, and gated areas (cell surface marker positive or negative) were compared in the histogram between the F-SU/GFP treated and untreated samples.

Northern blotting

Total RNAs were isolated from the cells by a Quick-Prep total RNA extraction kit (Amersham Pharmacia), and 4 μg of total RNAs were fractionated on an agarose gel (0.8% agarose, 1×MOPS, 18% formaldehyde). The gel was treated with 0.05 M NaOH and 10 mM NaCl for 15 min, and then with 0.1 M Tris-HCl (pH 7.4) for 20 min. The RNAs were transferred to positively charged nylon membranes (Boehringer Mannheim, Mannheim, Germany) and hybridized with a ^{32}P-labeled mCAT-1 probe, a 2.4 kb fragment derived from *Eco*RI digestion of pJET plasmid (a gift from J. M. Cunningham) [1]. Hybridization was performed for 20 h at 42 °C in 50% formamide, 5×SSC, 50 mM sodium phosphate, 1×Denhardt's solution, 0.1% SDS, and 50 μg/ml sermon sperm DNA. After hybridization, the membrane was washed 4 times for 5 min each at room temperature in 2×SSC-0.1% SDS, twice for 15 min each at 50 °C in 0.1×SSC-0.1% SDS, and finally twice for 5 min each at 50 °C in 2×SSC. Hybridization signals were detected by a bio-imaging analyzer (BAS 2000, Fujifilm). Concentration of the mCAT-1 mRNAs of NIH3T3 cells (a total of two or three mRNAs) and SIRC-NIH EcoR cells (a 2.7 kb mRNA) were estimated by standardizing with the ribosomal RNA content of each lane.

Results

Construction of the F-SU/GFP fusion protein

We prepared the F-SU/GFP soluble fusion protein consisting of a portion of the SU subunit of Friend MuLV Env and GFP using the baculovirus expression system. The F-SU/GFP included the entire receptor binding region located in the N-terminal half of the SU [2, 4, 5] but lacked the C-terminal 11 amino acids of the Friend SU. The GFP tag was introduced to detect readily and specifically the SU bound to cells by flow cytometry. One lot of culture supernatant of insect cells infected by the recombinant baculovirus was analyzed by Western blotting using anti-Rauscher MuLV gp70 antibody and anti-GFP antibody (Fig. 1). The

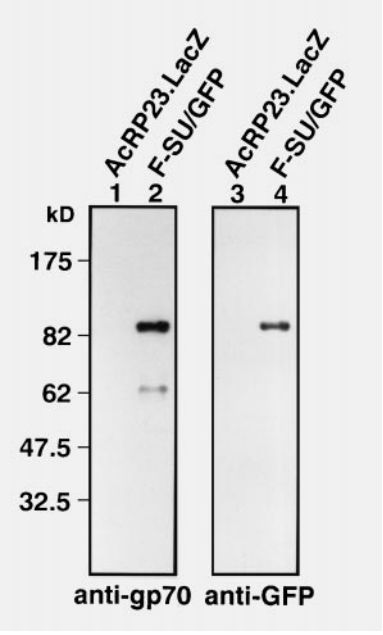

Fig. 1. The F-SU/GFP protein expressed in the culture supernatant of insect cells. Ten μl of serum-free culture supernatant of Tn5 cells infected with the recombinant baculovirus (F-SU/GFP) or parental virus (AcRP23.LacZ) were fractionated by 7.5% SDS-PAGE and transferred to a polyvinylidene difluoride membrane. The membrane was first probed with HRP-conjugated anti-GFP antibody (*3* and *4*), and after stripping the first probe, then with biotin-labeled anti-Rauscher MuLV gp70 antibody and HRP-conjugated streptavidin (*1* and *2*)

anti-gp70 antibody detected two bands with apparent molecular weights 87 kD and 63 kD (lane 2), while only the 87 kD band was detected by the anti-GFP antibody (lane 4). The expected molecular masses of the Friend SU portion and the GFP calculated by amino acid composition were 48 kD and 27 kD, indicating that the 87 kD molecule was the intact F-SU/GFP protein with about 12 kD of carbohydrates. The 63 kD molecule detected by only the anti-gp70 antibody might be a degradation product of the 87 kD.

Binding specificity of the F-SU/GFP fusion protein

The binding specificity of the F-SU/GFP was tested using cultured cell lines expressing or not expressing mCAT-1. The F-SU/GFP bound to mouse NIH3T3 cells expressing mCAT-1 but not to rabbit SIRC cells lacking mCAT-1 (Fig. 2). Rabbit SIRC cells transfected with the *mCAT-1* gene, SIRC-NIH EcoR [27], absorbed the F-SU/GFP (Fig. 2), indicating the mCAT-1-specific binding of the F-SU/GFP.

The F-SU/GFP did not bind to NIH3T3 cells persistently infected with Friend MuLV or FL21 cells which are NIH3T3 cells expressing Env protein derived from endogenous *Fv-4*^{r} ecotropic MuLV [18] (Fig. 2). The resistance of these cells to the F-SU/GFP-binding may be correlated to the resistance of these cells to Eco-MuLV infection. The F-SU/GFP was almost identical to the soluble naturally occurring ecotropic *Fv-4*^{r} SU protein with regard to the binding specificity and the flow cytometric pattern [9].

SIRC-NIH EcoR cells showed about 5-fold higher F-SU/GFP absorbing capacity than NIH3T3 cells (Fig. 2). NIH 3T3 cells expressed two or three mCAT-1 mRNAs ranging 6.0 to 8 kb (Fig. 3, lane 1) [1, 12], while SIRC-NIH EcoR cells

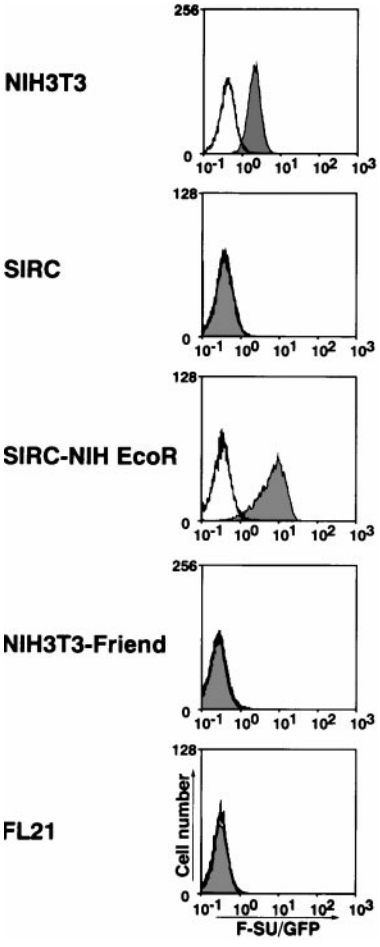

Fig. 2. Binding of the F-SU/GFP to mCAT-1. Mouse NIH3T3 cells (expressing mCAT-1), rabbit SIRC cells (lacking mCAT-1), rabbit SIRC-NIH EcoR cells (SIRC cells transfected with the *mCAT-1* gene), Friend MuLV-infected NIH3T3 cells and FL21 cells (NIH3T3 cells expressing endogenous *Fv-4*^{r} Eco-MuLV Env) were incubated with (closed histograms) or without (open histograms) the F-SU/GFP. Cells were analyzed by flow cytometry

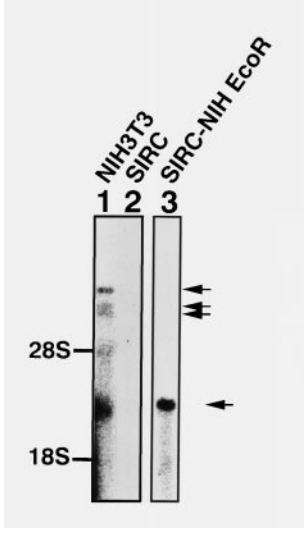

Fig. 3. RNA expression of mCAT-1 in NIH3T3, SIRC, and SIRC-NIH EcoR cells. RNAs isolated from NIH3T3 (*1*), SIRC (*2*), and SIRC-NIH EcoR (*3*) cells were fractionated on 0.8% agarose gel including formaldehyde and hybridized with the ^{32}P-labeled mCAT-1 cDNA probe. The migration positions of 28S (4.8 kb) and 18S (1.9 kb) ribosomal RNA are indicated at the left side of the panel. Right-side arrows indicate 6.0–8 kb endogenous mCAT-1 RNAs and a 2.7 kb mCAT-1 RNA. The signals from NIH3T3 and SIRC cells were detected by exposure of an imaging plate for 4 days, and that of SIRC-NIH EcoR cells was detected after exposure for 3.5 h

transfected with the mCAT-1 cDNA expressed a 2.7 kb mRNA that was more abundant (more than 50-fold) than the endogenous RNA transcripts (lane 3).

To enhance the intensity of fluorescence, the F-SU/GFP-bound cells were treated with a rabbit anti-GFP antibody and an FITC-labeled anti-rabbit IgG antibody. These treatments enhanced the fluorescence signals of the F-SU/GFP-bound NIH3T3 and SIRC-NIH EcoR cells about 10-fold but did not alter the histogram patterns (data not shown). The fluorescence was not changed when these cells were not treated with the F-SU/GFP, suggesting that the antibody treatment specifically enhanced the detection sensitivity of the F-SU/GFP bound to cells. Binding of the F-SU/GFP to SIRC cells was not detected even if the sensitivity was increased.

Binding of the F-SU/GFP to BALB/c cells

We measured the expression level of the Eco-MuLV receptor on the surface of mouse hematopoietic cells. Cells from BALB/c, susceptible to Eco-MuLV infection, were separated into subpopulations according to the cell surface markers: TER119 and Mac-1 for BM cells, CD3 for thymocytes, and Thy1 and sIgM for spleen cells. The $TER119^+$ and the $Mac\text{-}1^+$ subpopulations in BM cells and $Thy1^+$ and $sIgM^+$ subpopulations in spleen cells were exclusive. Each subpopulation was gated and analyzed by histograms (Fig. 4). All these subpopulations absorbed the F-SU/GFP to various degrees (Fig. 4).

Binding of the F-SU/GFP was particularly evident in the $TER119^+$ BM subpopulation; these cells homogeneously absorbed a high amount of the F-SU/GFP (about 11 mean fluorescence intensity) (Fig. 4). The IF intensity of the F-SU/GFP-treated cells should be the sum of that of untreated cells (background) and that of cell-bound F-SU/GFP plus rabbit anti-GFP/FITC-labeled anti-rabbit IgG. The background of $TER119^+$ cells was about 2, so the putative real fluorescence should be 9. When the same calculation was done for the other subpopulations, none of them showed more than 3. Because the $TER119^+$ cells are generally Mac-1-negative, there was a small fraction of cells in the $Mac\text{-}1^-$ subpopulation absorbing as high an amount of the F-SU/GFP as the $TER119^+$ cells (Fig. 4). The TER119 antigen is expressed on the erythroid lineage, exclusively on mature erythroblasts and erythrocytes but not on immature BFU-E and CFU-E [10].

Binding of the F-SU/GFP to cells from C4W and AKR mice

C4W and AKR mice both express Env glycoproteins of endogenous Eco-MuLVs, while C4W mice are resistant [6, 22] and AKR mice are susceptible [25] to exogenous Eco-MuLV infection. We analyzed these mice to assess whether the different susceptibilities to Eco-MuLV were correlated to the SU absorbing capacities of the hematopoietic cells. In contrast to BALB/c cells, both C4W and AKR cells were strongly resistant to the F-SU/GFP-binding (Fig. 5). Thus, we were unable to detect any obvious difference in the F-SU/GFP-binding capacity between C4W and AKR mice.

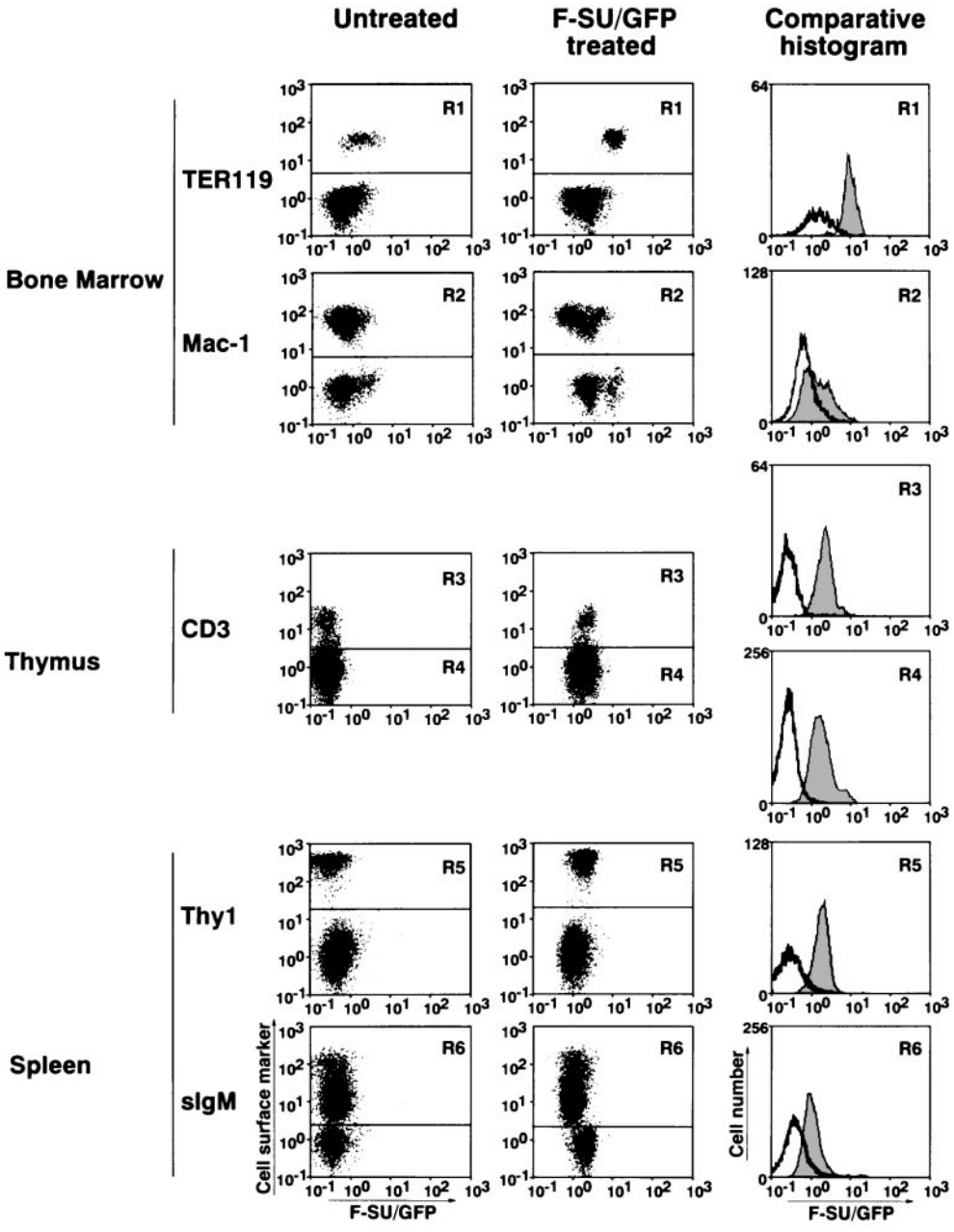

Fig. 4. Binding of the F-SU/GFP to hematopoietic cells from BALB/c mice. Cells from the bone marrow, thymus, and spleen of BALB/c mice were incubated without or with 150 μl of the F-SU/GFP solution, then stained with rabbit anti-GFP antibody and FITC-labeled anti-rabbit IgG antibody. The cells were also treated with either antibody against the cell surface markers shown on the left of each panel. All the samples were analyzed under the same setting; voltages for FL1 and FL2 were 625 and 998, gains for FL1 and FL2 were 1.0 and 1.0, compensations of FL1-%FL2 and FL2-%FL1 were 0.5 and 51.0. Dot plots (left panels) showed cells untreated (background) or treated with the F-SU/GFP. The fluorescence intensities of the gated populations (R1–R6) were compared by histograms (right panels). The cells incubated with the F-SU/GFP were indicated by closed histograms, and the cells incubated without the F-SU/GFP were indicated by open histograms

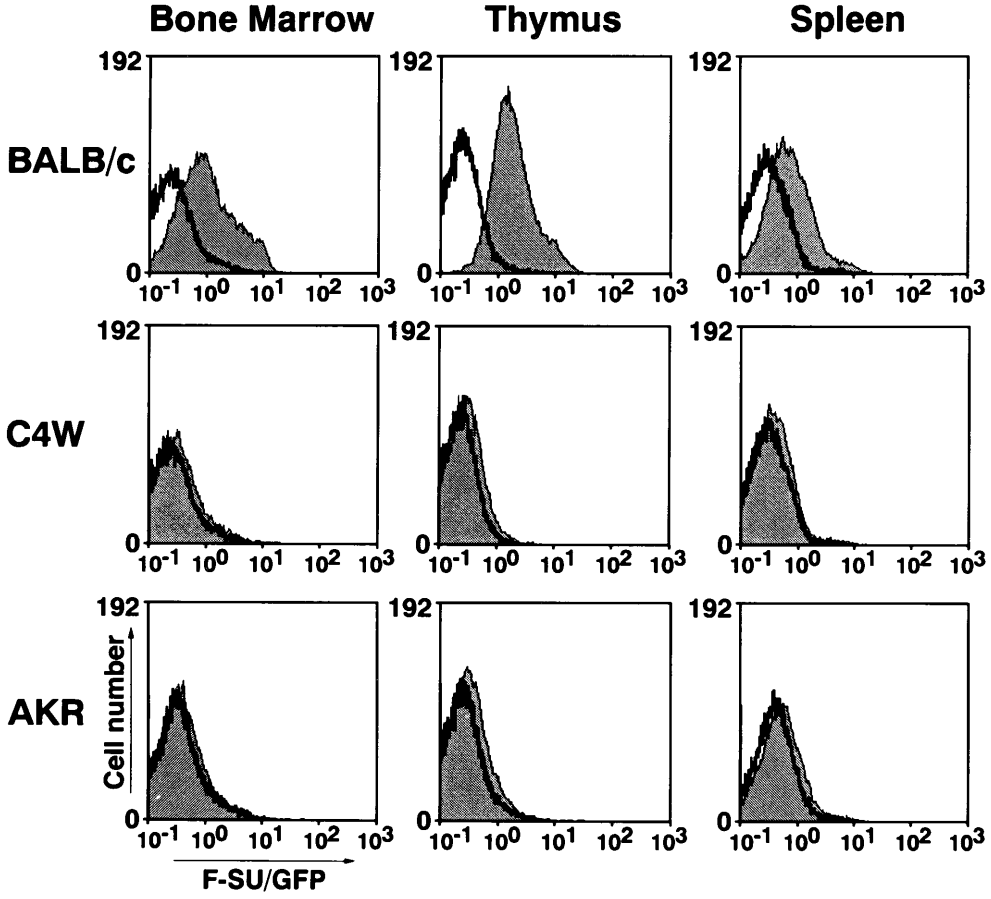

Fig. 5. Comparison of the F-SU/GFP-absorbing capacity of hematopoietic cells from BALB/c, C4W and AKR mice. Cells from the bone marrow, thymus and spleen of BALB/c, C4W and AKR mice were incubated without (open histograms) or with (closed histograms) 40 μl of the F-SU/GFP solution, then stained with rabbit anti-GFP antibody and FITC-labeled anti-rabbit IgG antibody. The cells were analyzed without the separation by the cell surface markers

Discussion

We prepared the F-SU/GFP fusion protein to quantify the capacities of hematopoietic cells to absorb exogenous viral SU. The protein was expressed in insect cells by the baculovirus system. Amphotropic and ecotropic MuLV Env proteins expressed by the baculovirus system were also studied by others, and the proper receptor binding specificities were demonstrated in all of the studies [3, 4]. It is often difficult to distinguish exogenous and endogenous virus antigens when antibodies against viral proteins are used. The GFP tag can overcome the problem without losing the receptor binding specificity of the fusion protein (Fig. 2). The F-SU/GFP produced by insect cells showed the receptor binding specificities (Fig. 2) to be indistinguishable from those of the *Fv-4*^{r} ecotropic SU produced in mice [9].

This study demonstrated that the soluble fusion protein F-SU/GFP bound to all the subpopulations of the hematopoietic cells from BALB/c mice that are susceptible to ecotropic MuLV infection (Fig. 4). The most obvious binding was found in the TER119$^+$ BM subpopulation. This subpopulation showed a high and homogeneous SU-absorbing capacity (Fig. 4). The TER119$^+$ antigen is expressed on the erythroid lineage from early erythroblasts through mature erythrocytes, but not on the more primitive cells with BFU-E or CFU-E activities [10]. Our data may be consistent with the abnormality seen in *mCAT-1* knockout mice, which died on the day of birth with anemia [23]. Compared with wild-type mice, the knockout mice had a normal number of BFU-E and CFU-E in the fetal liver but a decreased number of erythroid progenitors and mature red blood cells [23]. Thus, cells at the late stage of erythropoiesis have a higher SU-absorbing capacity possibly due to a high expression of functional mCAT-1 receptor in the normal mice, and, in the *mCAT-1* knockout mice, could be abnormal or deficient. Friend leukemia virus induces abnormal proliferation of erythroid cells and erythroleukemia, but this target cell specificity may be unrelated to the high mCAT-1 expression of the TER119$^+$ erythroblasts. The proliferating cells are considered to be the erythroid progenitor cells, such as BFU-E and CFU-E, probably not expressing TER119 [10]. The erythroid-specific growth is a consequence of activation of the erythropoietin receptor expressed in erythroid progenitor cells by a rearranged SU encoded by spleen focus forming virus of the Friend leukemia virus complex [17, 26].

In addition to cells with a high SU-absorbing capacity, cells with a lower but significant SU-absorbing capacity were found in most hematopoietic subpopulations (Fig. 4). The expression level of mCAT-1 receptors may not be proportional to the susceptibility to virus, because in cultured non-mouse cell lines transfected with the *mCAT-1* gene, the SU-absorbing capacity did not linearly correspond to the degree of susceptibility of the cells to Eco-MuLV infection [9, 28]. A low level of mCAT-1 expression could be sufficient to confer susceptibility to infection and correlate the susceptibility of a wide range of hematopoietic cells to Eco-MuLV infection. Although the physiological importance of this lower expression of mCAT-1 is unknown, it might be related to other abnormalities found in the *mCAT-1* knockout mice, including reduced body size and possible defects in the generation of white blood cells [23].

Of the 4 members of the cationic amino acid transporter gene family, *CAT-1*, *CAT-2*, *CAT-3* and *CAT-4*, only mouse *mCAT-1* and rat *rCAT-3* genes have been shown to facilitate the Eco-MuLV infection [20]. The mouse *mCAT-3* gene is exclusively expressed in the brain [11] but has not been tested for its function as a receptor for Eco-MuLV. Because embryo fibroblast cells from *mCAT-1* knockout mouse were resistant to Eco-MuLV infection [23], mCAT-1 is probably the major functional receptor for Eco-MuLV in vivo. However, our experiments do not rule out the possibility that the F-SU/GFP binds to molecules other than mCAT-1.

The binding of the F-SU/GFP to C4W cells was strongly inhibited (Fig. 5). This is probably due to the occupation of receptors by the Env protein

of endogenous *Fv-4*^{r} Eco-MuLV. The Env protein was detected in all subpopulations of C4W hematopoietic cells (data not shown) and in various organs [7, 15]. Cells persistently infected with Friend MuLV or expressing endogenous *Fv-4*^{r} Eco-MuLV Env did not absorb the F-SU/GFP (Fig. 2), suggesting the occupation of receptors by Env proteins expressed in the cells. Although AKR mice have not been systematically tested for their expression pattern of endogenous Env proteins, the reduced absorbing capacity of AKR cells suggested that most AKR hematopoietic cells were infected with endogenous Eco-MuLV. Eco-MuLV could replicate well in the spleen, BM, thymus, and lymph node cells in BALB/c mice, while in C4W mice the replication was strongly restricted in all of these tissues [7]. AKR mice are susceptible to infection by Eco-MuLVs such as Friend virus and Moloney sarcoma virus. Although C4W and AKR mice have different susceptibilities to Eco-MuLV infection, they did not show any obvious difference in the SU-absorbing capacity of each subpopulation (Fig. 5).

Co-expression of mCAT-1 and Eco-MuLV Env reduced the mCAT-1 transport activity and mCAT-1 glycosylation [13, 29]. Although most hematopoietic cells of C4W and probably AKR mice express Eco-MuLV Env, no functional abnormalities have been found in the hematopoietic cells. It is interesting to determine the quantity and functional activity of mCAT-1 in mice that constitutively express the endogenous viral Env glycoprotein.

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