Proteolytic Conversion of STAT3α to STAT3γ in Human Neutrophils

ROLE OF GRANULE-DERIVED SERINE PROTEASES

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Four isoforms (α, β, γ, and δ) have been identified for signal transducer and activator of transcription 3 (STAT3). It has been reported that STAT3γ, which is derived from STAT3α by limited proteolysis during granulocytic differentiation, is a major STAT3 isoform expressed in human neutrophils. We confirmed that STAT3γ was a major STAT3 isoform detected in human neutrophil lysates prepared with the conventional lysis buffer. The enzymes capable of converting STAT3α to STAT3γ in vitro were localized in neutrophil granule fraction and were released into the medium upon ionomycin stimulation. The enzyme activity was strongly inhibited by phenylmethylsulfonyl fluoride, CuSO4, and ONO-5046 (a specific inhibitor of neutrophil elastase), but not by aprotinin, leupeptin, benzamidine, and EDTA. STAT3γ was effectively generated in vitro from STAT3α by limited proteolysis with human neutrophil elastase or protease 3 but not cathepsin G. The converting activity in neutrophil lysates was reduced by immunodepletion of elastase but not proteinase 3. Unexpectedly, STAT3γ was undetected in the lysates of neutrophil-derived cytoplasts, which lack granules, and the cytosol fraction prepared by nitrogen cavitation. The STAT3 isoform detected in these preparations was primarily STAT3α. STAT3γ was also undetected in the lysates of PMSF-potentiated neutrophils and was markedly decreased in the lysates of ionomycin-potentiated neutrophils. These findings indicate that, in contrast to the previous reports, STAT3α, but not STAT3γ, is primarily expressed in human neutrophils, and STAT3γ is rapidly generated from STAT3α by limited proteolysis with granule-derived serine proteases during preparation of neutrophil lysates with the conventional lysis buffer.

Signal transducer and activator of transcription 3 (STAT3),1 a transcription factor, participates in a wide variety of physiological processes (1). In myeloid cells, STAT3 has been implicated in regulating cellular processes (1). In myeloid cells, STAT3 has been implicated in a wide variety of physiological processes (1). In myeloid cells, STAT3 has been implicated in regulating cellular processes (1).

Experimental Procedures

Materials—Human neutrophil elastase and cathepsin G were purchased from Calbiochem-Novabiochem (San Diego, CA). Human neutrophil protease 3 was purchased from Elastin Products Co., Inc. (Owensville, MO). Phenylmethylsulfonyl fluoride (PMSF), aprotinin, leupeptin, benzamidine, cytochalasin B, ionomycin, and Ficoll-70 were purchased from Sigma. Conray was purchased from Mallinkrodt (St. Louis, MO). Picoll was purchased from Pharmacia Fine Chemicals (Piscataway, NJ), and ONO-5046, a specific inhibitor of neutrophil elastase (13), was provided by Ono Pharmaceutical Co. Ltd. (Osaka, Japan). Rabbit polyclonal antibody against STAT3, which recognizes the N-terminal portion of STAT3, and goat anti-rabbit IgG antibody conjugated with horseradish peroxidase were purchased from Cell Signaling Technology (Beverly, MA). Rabbit polyclonal antibody against human neutrophil elastase was purchased from Athens Research Technologies (Athens, GA), goat polyclonal antibody against human proteinase 3 purchased from Cell Systems, Inc. (Bothell, WA). Rat monoclonal antibody against Mcl-1 was purchased from BD Biosciences (San Jose, CA).

1 The abbreviations used are: STAT3, signal transducer and activator of transcription 3; PMSF, phenylmethylsulfonyl fluoride; HEPES, N-2-hydroxyethyl-piperazine-N’-2-ethane-sulfonic acid; PIPES, piperazine-N,N’-bis(2-ethanesulfonic acid).

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ase 3 was purchased from Santa Cruz Biotechnology (Santa Cruz, CA), rabbit anti-goat IgG antibody conjugated with hors eradish peroxidase was purchased from DAKO (Glostrup, Denmark), and protein G-Sepharose 4 Fast Flow and the enhanced chemiluminescence (ECL) Western blotting system were purchased from Amersham Pharmacia Biotech (Buckinghamshire, England).

Preparation of Cells—Human neutrophils and mononuclear cells were prepared from healthy adult donors as described previously (9), using dextran sedimentation, centrifugation with Conray-Ficoll, and hypotonic lysis of contaminated erythrocytes. Neutrophil fractions contained >98% neutrophils. Lymphocytes were further purified from mononuclear cells by centrifugal elutriation in a Hitachi SRR6Y elutriation rotor (Hitachi, Tokyo, Japan) (14). Lymphocyte fractions contained >98% lymphocytes. Cells were suspended in Hank’s balanced salt solution containing 10 mM HEPES, pH 7.4.

Preparation of Cytoplasts—Cytoplasts were prepared from 1 × 10⁶ neutrophils as described previously (15). Briefly, neutrophils were centrifuged through a discontinuous Ficol1-70 gradient (12.5, 16, and 25%) prewarmed to 37°C and containing 0.5 μg/ml cytochalasin B. Centrifugation was performed for 30 min at 34°C in a Hitachi CP100L ultracentrifuge with a P28S2 swing rotor (Hitachi, Tokyo) at 81,000 × g. After centrifugation, the top band of cellular material was collected. This band was composed of >99% cytoplasts, as assessed by light microscopy of cytoplast preparations stained with May-Grunwald-Giemsa. Cytoplasts were recognized by their absence of a nucleus. Cytoplasts were washed with phosphate-buffered saline (PBS) and suspended in Hank’s balanced salt solution containing 10 mM HEPES, pH 7.4 (2.10⁶ cytoplasts/ml).

Western Blotting—Western blotting was performed as described previously (9). Cells were suspended in ice-cold lysis buffer containing 50 mM PIPES, pH 7.0, 0.5% Triton X-100, 50 mM KCl, 10 mM EGTA, 2 mM MgCl₂, 1 mM sodium orthovanadate, and 5 mM sodium fluoride. The reaction mixture equivalent to 1 × 10⁶ cells was mixed with each other. When required, neutrophil lysates were replaced by other samples such as the cytosol fraction and the lysates of cytoplasts and granule fraction. The reaction mixtures were incubated for 15–60 min at 37°C, and then, the immunoblotting was performed using the anti-STAT3 antibody.

Immunodepletion of Elastase and Proteinase 3 from Neutrophil Lysates—Neutrophil lysates and lymphocyte lysates prepared with the protease inhibitor-free lysis buffer were mixed at 1:50 on the basis of cell number; i.e. 0.5 μl of neutrophil lysates equivalent to 5 × 10⁵ cells and 25 μl of lymphocyte lysates equivalent to 2.5 × 10⁶ cells were mixed with each other. When required, neutrophil lysates were replaced by other samples such as the cytosol fraction and the lysates of cytoplasts and granule fraction. The reaction mixtures were incubated for 15–60 min at 37°C, and then, the immunoblotting was performed using the anti-STAT3 antibody.

Treatment of Neutrophils with PMSF or Ionomycin—PMSF- or ionomycin-pretreated neutrophils were prepared by treatment of neutrophils with PMSF (1 mM) for 1 h or ionomycin (1 μM) for 30 min at 37°C. PMSF- or ionomycin-pretreated neutrophils were washed with PBS and lysed with the conventional lysis buffer. The cell-free supernatant from ionomycin-stimulated neutrophils was used as granule enzymes released into the medium upon ionomycin stimulation.

Disruption of Neutrophils by Nitrogen Cavitation—Disruption of neutrophils by nitrogen cavitation was performed as described previously (16). Briefly, neutrophils (1 × 10⁶ cells/ml) were suspended in the disruption buffer containing 10 mM PIPES (pH 7.2), 100 mM KCl, 3 mM NaCl, 3.5 mM MgCl₂, 1 mM ATP, 1 mM sodium orthovanadate, and 5 mM sodium fluoride and were pressurized with nitrogen for 5 min at 380 psi (pounds per square inch) in a nitrogen bomb (Parr Instrument, Moline, IL) at 4°C. After release from the cavitation bomb, the cavitate was collected dropwise into EGTA to a final concentration of 1.5 mM. Disrupted cells were centrifuged for 15 min at 400 × g to obtain the nuclear fraction. The postnuclear supernatant was used as crude neutrophil lysates. The reaction mixtures were incubated for 15 min at 18,000 × g to obtain the granule and cytosol fractions. The granular and cytosolic fractions were lysed with the protease inhibitor-free lysis buffer and centrifuged for 5 min at 18,000 × g to obtain the lysates. By this method, most activity (>95%) of elastase, an enzyme in the primary granule, was detected in the granule fraction when elastase activity was determined using methoxysuccinil-Ala-Ala-Pro-Val-p-nitroanilide as a substrate (17).

RESULTS

Proteolytic Conversion of STAT3α to STAT3γ by Neutrophil Lysates—Neutrophil and lymphocyte lysates were prepared with the conventional lysis buffer containing various protease inhibitors, and STAT3 isoforms were analyzed by immunoblotting with anti-STAT3 antibody, which recognizes the N-terminal portion of STAT3. As shown in Fig. 1A, three bands were detected in neutrophil lysates. Each band may correspond to STAT3α, STAT3β, and STAT3γ on the basis of molecular mass (4, 10, 12). In lymphocyte lysates, one band, which corresponds...
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Fig. 2. The converting activity is localized in the granule fraction. A, neutrophils were disrupted by nitrogen cavitation, and disrupted samples were separated into the cytosol, nuclear, and granule fractions (Fr). The nuclear and granule fractions, neutrophils, and lymphocytes were lysed with the protease inhibitor-free lysis buffer. Each sample (0.5 μl) was mixed with lymphocyte lysates (25 μl) equivalent to 2.5 × 10^6 cells. The reaction mixtures were incubated for 30 min at 37 °C, followed by immunoblotting with anti-STAT3 antibody. As a control, lymphocyte lysates alone were incubated for 30 min at 37 °C. The reaction mixture equivalent to 1 × 10^6 cells was loaded onto each lane. The results shown are representative of eight independent experiments.

to STAT3α, was detected. If STAT3γ were produced by limited proteolysis of STAT3α in intact neutrophils (12), neutrophil lysates would contain proteases capable of converting STAT3α to STAT3γ. To demonstrate this activity, neutrophil and lymphocyte lysates prepared with the protease inhibitor-free lysis buffer were mixed to each other, and the mixtures were incubated for 15–60 min at 37 °C, followed by immunoblotting with anti-STAT3 antibody. As shown in Fig. 1A, STAT3α was cleaved within 15 min after incubation of the mixtures. The cleavage of STAT3α was accompanied with the concomitant appearance of lower molecular mass bands of ~83 and 72 kDa, which apparently correspond to STAT3β and STAT3γ, respectively. Incubation of lymphocyte lysates alone resulted in no cleavage of STAT3α. These findings indicate that neutrophil, but not lymphocyte, lysates contain proteases, which can mediate the conversion of STAT3α to STAT3γ. The converting activity was markedly inhibited by PMSF, but not inhibited by aprotinin, leupeptin, benzamidine, and EDTA (Fig. 1B), suggesting that the conversion of STAT3α to STAT3γ may be mediated by certain serine proteases.

The Converting Activity Is Localized in the Granule Fraction—Neutrophils were disrupted by nitrogen cavitation, and disrupted samples were separated into the cytosol, nuclear, and granule fractions. Each fraction was analyzed for the activity of converting STAT3α to STAT3γ. Strong converting activity was detected in the granule fraction, which gave the proteolytic profile of STAT3α identical to that obtained with whole neutrophil lysates (Fig. 2A). The nuclear fraction showed a slight converting activity, presumably because of contaminated granules and/or undisrupted neutrophils. Unexpectedly, the converting activity was undetected in the cytosol fraction. These findings indicate that the converting activity is primarily localized in neutrophil granules but not in the cytosol.

To obtain additional evidence that the converting enzymes are localized in granules, neutrophils were stimulated with ionomycin to release granule enzymes into the medium, and the converting activity in the medium was analyzed. As shown in Fig. 2B, the converting activity was detected in the cell-free supernatant from ionomycin-stimulated neutrophils but not that from unstimulated control cells. These findings also suggest that the converting enzymes are localized in granules and released upon ionomycin stimulation.

Conversion of STAT3α to STAT3γ by Granule-derived Serine Proteases—The results depicted in Figs. 1 and 2 suggest that certain serine proteases in granules are responsible for the conversion of STAT3α to STAT3γ. The major serine proteases in human neutrophil granules are elastase, cathepsin G, and proteinase 3. The possible conversion of STAT3α to STAT3γ by these proteases was assessed by adding purified human neutrophil elastase, cathepsin G, and proteinase 3 to lymphocyte lysates; the reaction mixtures were incubated for 30 min at 37 °C, followed by immunoblotting with anti-STAT3 antibody. As shown in Fig. 3A, STAT3α was cleaved by elastase or proteinase 3, and the cleavage of STAT3α was accompanied by the concomitant appearance of lower molecular mass bands of ~83 and 72 kDa, apparently corresponding to STAT3β and STAT3γ, respectively. Elastase was more potent than proteinase 3 in this effect. On the other hand, STAT3α was rapidly degraded by cathepsin G, leaving no band detectable by anti-STAT3 antibody. These findings suggest that elastase and proteinase 3 could contribute to the conversion of STAT3α to STAT3γ, with elastase being more potent than proteinase 3. To determine whether elastase participates in the conversion of STAT3α to STAT3γ in the cell-free system composed of neutrophil and lymphocyte lysates, the effects of Cu^2+ (a potent inhibitor of elastase; Ref. 18) and ONO-5046 (a specific inhibitor of neutrophil elastase; Ref. 13) were studied. As shown in Fig. 3B, the conversion of STAT3α to STAT3γ was completely abolished by Cu^2+ and was markedly inhibited by ONO-5046, suggesting that elastase plays a major role in the conversion of STAT3α to STAT3γ in this cell-free system. This notion was also supported by the finding that the converting activity in neutrophil lysates was reduced when elastase, but not proteinase 3, was immunodepleted from neutrophil lysates by using the antibody-coated beads (Fig. 3C).

Absence of STAT3γ in the Cytosol Fraction and Cytoplasts—STAT3γ was consistently detected in whole neutrophil lysates prepared with the conventional lysis buffer containing various protease inhibitors (Figs. 1–3; Refs. 4, 9, 12). However, the results depicted in Figs. 1–3 suggest that granule enzymes participate in the appearance of STAT3γ despite the fact that STAT3 is localized primarily in the cytoplasm of resting cells. These findings raise the possibility that STAT3α might be highly susceptible to the proteolytic cleavage by granule enzymes, and STAT3γ might be generated solely from STAT3α during preparation of neutrophil lysates. In fact, as shown in Fig. 4, STAT3γ was undetected in the cytosol fraction prepared by nitrogen cavitation. The cytosol fraction prepared by this method lacks granules, and most activity (> 95%) of elastase, a primary granule enzyme, was detected in the granule fraction (data not shown). The STAT3 isofrom detected in the cytosol fraction was primarily STAT3α, with a faint band of STAT3β (Fig. 4). To obtain additional evidence, we prepared neutrophil-derived cytoplasts, which lack a nucleus, granules, and mitochondria (15). STAT3γ was also undetected in cytoplast lysates. The STAT3 isofrom detected in cytoplast lysates was primarily STAT3α, with a faint band of STAT3β, a profile identical to that observed in the cytosol.
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Fig. 3. Proteolytic conversion of STAT3α to STAT3γ by neutrophil elastase and proteinase 3. A, human neutrophil elastase, cathepsin G, or proteinase 3 (1 μl of 0.2, 0.6, or 2 milliunits/μl, respectively, for each protease) was mixed with lymphocyte lysates (10 μl) equivalent to 1 × 10⁶ cells. The reaction mixtures were incubated for 30 min at 37 °C, followed by immunoblotting with anti-STAT3 antibody. One unit of these proteases is defined as the amount that will hydrolyze 1 μmol of the substrate per min at 25 °C, when methoxy succinyl-Ala-Ala-Pro-Val-p-nitroanilide, succinyl-Ala-Ala-Pro-Phe-p-nitroanilide, and t-butyloxycarbonyl-Ala-Ala-norvalyl-thiobenzyl ester are used as the substrate for elastase, cathepsin G, and proteinase 3, respectively. The results shown are representative of four independent experiments. B, reaction mixtures containing neutrophil and lymphocyte lysates described in Fig. 1 were incubated in the presence or absence of CuSO₄ (10 μM) or ONO-5046 (10 μM) for 30 min at 37 °C, followed by immunoblotting with anti-STAT3 antibody. As a control, lymphocyte lysates alone were incubated for 30 min at 37 °C. The results shown are representative of three independent experiments. C, elastase and proteinase 3 were immunodepleted from neutrophil lysates (20 μl) equivalent to 2 × 10⁶ cells by using the beads coupled with anti-elastase or anti-proteinase 3 antibody. Control samples were prepared by incubating neutrophil lysates with the beads uncoupled with any antibody. Upper panel, the elastase-depleted sample (0.4 μl), the proteinase 3-depleted sample (1 μl), or the control sample was mixed with lymphocyte lysates (20 μl) equivalent to 2 × 10⁶ cells. The reaction mixtures were incubated for 30 min at 37 °C, followed by immunoblotting with anti-STAT3 antibody. As a control, lymphocyte lysates alone were incubated for 30 min at 37 °C. Lower panel, to confirm the depletion of elastase and proteinase 3 from neutrophil lysates, the elastase-depleted sample, proteinase 3-depleted sample (10 μl), or the control sample (10 μl) was analyzed by immunoblotting with anti-elastase or anti-proteinase 3 antibody. The results shown are representative of three independent experiments.

Fig. 4. Absence of STAT3γ in the cytosol fraction and neutrophil-derived cytoplasts and decreased level of STAT3γ in PMSF- and ionomycin-pretreated neutrophils. Neutrophils were pre-treated with PMSF (1 mM) for 1 h or ionomycin (1 μM) for 30 min at 37 °C, washed with PBS, and lysed with the conventional lysis buffer. Neutrophil-derived cytoplasts and untreated control neutrophils were lysed with the conventional lysis buffer. The cytosol fraction was prepared by nitrogen cavitation. PMSF- and ionomycin-pretreated neutrophil lysates equivalent to 1 × 10⁶ cells, cytoplast lysates equivalent to 2 × 10⁶ cytoplasts, the cytosol fraction equivalent to 1 × 10⁶ cells, and untreated control neutrophil lysates equivalent to 1 × 10⁶ cells were loaded onto each lane. The immunoblotting was performed using anti-STAT3 antibody. The results shown are representative of three independent experiments.

Discussion

It has been reported that STAT3γ is a major STAT3 isoform expressed in human neutrophils and generated from STAT3α by limited proteolysis during differentiation into mature neutrophils (4, 9, 10, 12). In fact, STAT3γ is a major STAT3 isoform phosphorylated in human neutrophils stimulated by G-CSF or GM-CSF, and STAT3 activation has been demonstrated to be involved in G-CSF- or GM-CSF-mediated prolongation of neutrophil survival (9, 19). The appearance of STAT3γ during granulocytic differentiation also suggests a possible role of STAT3γ in the regulation of granulocytic differentiation (4). These previous observations strongly indicate that STAT3γ may play an important role in the functional regulation of myeloid cells and prompted us to identify the proteolytic enzymes responsible for conversion of STAT3α to STAT3γ. The results presented here show that, unexpectedly, STAT3γ is absent in intact human neutrophils, and the major isoform expressed in human neutrophils is STAT3α, suggesting that the STAT3 isoform involved in neutrophil functions is primarily STAT3α and not STAT3γ. In addition, the results show that STAT3α is highly susceptible to proteolytic cleavage by neutrophil granule-derived serine proteases, and both STAT3β and STAT3γ are rapidly generated from STAT3α by limited proteolysis with granule-derived proteases, especially elastase, during the preparation of neutrophil lysates with the conventional lysis buffer. These findings indicate that careful interpretation and analysis should be performed to determine the role and expression profile of STAT3 isoform in myeloid cells.

The absence of STAT3γ in intact human neutrophils is supported by the findings that STAT3γ was undetected in cytoplast lysates and the cytosol fraction prepared by nitrogen cavitation. Instead, abundant STAT3α was detected in cytoplast lysates and the cytosol fraction. In addition, the cytosol fraction did not contain the activity for conversion of STAT3α to STAT3γ in the cell-free system. These findings suggest that STAT3α is highly susceptible to proteolytic cleavage by certain neutrophil proteases and is rapidly cleaved to generate
STAT3γ during the preparation of neutrophil lysates with the conventional lysis buffer. Consistent with this is the finding that STAT3γ was undetected in the lysates of PMSF-pre-treated neutrophils. The high susceptibility of STAT3α to limited proteolysis by neutrophil-derived proteases is also supported by the finding that other proteins such as extracellular signal-regulated kinase and p38 mitogen-activated protein kinase are not cleaved under the same conditions (20, 21).

The activity for conversion of STAT3α to STAT3γ in the cell-free system was detected in neutrophil lysates but not in lymphocyte lysates. The profile of cleavage products of STAT3α obtained in the cell-free system was identical to that of STAT3 isoforms detected in whole neutrophil lysates. The fractionation study revealed that the converting activity was detected solely in the granule fraction. The converting activity was also detected in the cell-free supernatant from ionomycin-stimulated neutrophils, with a concomitant decrease in its activity in cells, indicating that the converting enzymes could be released to the extracellular milieu upon ionomycin stimulation. All of these findings indicate that granule enzymes are responsible for the conversion of STAT3α to STAT3γ. Among granule enzymes studied, purified human neutrophil elastase and proteinase 3, but not cathepsin G, were found to cleave STAT3α to generate STAT3γ, when assessed on the basis of molecular mass. Elastase was more potent than proteinase 3 in this effect, and the converting activity in neutrophil lysates was reduced by immunodepletion of elastase but not proteinase 3. These findings and the remarkable inhibition of conversion of STAT3α to STAT3γ by Cu²⁺ and ONO-5046 taken together indicate that elastase may be primarily responsible for the conversion of STAT3α to STAT3γ. The appearance of STAT3γ during granulocytic differentiation (4, 12) may be explained by the synthesis of primary granule enzymes, including elastase, during granulocytic differentiation, which may generate STAT3γ from STAT3α during the preparation of cell lysates with the conventional lysis buffer.

It has been reported that STAT3β is expressed in leukemic blasts from most patients (~80%) with acute myelogenous leukemia (AML) and constitutive activation of STAT3β is associated with short disease-free survival (22, 23). In these previous studies, the expression of STAT3β was analyzed using whole-cell lysates, and STAT3β in AML blasts was found to be generated primarily from STAT3α by limited proteolysis with PMSF-sensitive serine proteases but not by alternative mRNA splicing (22). These findings raise the possibility that STAT3β detected in AML blasts might be, at least in part, generated from STAT3α by limited proteolysis with granule enzymes during the preparation of cell lysates, as demonstrated in the present experiments with human neutrophils. Further detailed analysis is required to conclude that a large amount of STAT3β is, in fact, expressed in intact leukemic blasts in some cases, although it is possible that the processing of STAT3α may be dysregulated in certain AML cases. In addition, it should be determined whether STAT3β derived from proteolysis of STAT3α exhibits the same function as STAT3β derived from alternative mRNA splicing. In this regard, it is of interest that neutrophil elastase cleaves promyelocytic leukemia-retinoic acid receptor (PML-RARα) and is important for the development of acute promyelocytic leukemia in mice, although it is unknown how elastase in primary granules could encounter PML-RARα, which exists in a nucleus (24). In any case, the present experiments demonstrate that STAT3α, but not STAT3γ, is primarily expressed in human neutrophils, and STAT3α, but not STAT3γ, may play a role in the regulation of neutrophil functions such as neutrophil survival.

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REFERENCES
1. Levy, D. E., and Lee, C. (2002) J. Clin. Invest. 109, 1143–1148
2. McLemore, M., Grewal, S., Liu, F., Archambault, A., Poursine-Laurent, J., Haug, J., and Link, D. C. (2001) Immunity 14, 193–204
3. Lee, C., Zay, R., Gimeno, R., Gertner, R., Westphal, B., Takeshita, K., DePinho, R. A., and Levy, D. E. (2002) Immunity 17, 63–72
4. Hevehan, D. L., Miller, W. M., and Papoutsakis, E. T. (2002) Blood 99, 1627–1637
5. Takada, K., Claesen, B. E., Kaisho, T., Toujimura, T., Terada, N., Forster, I., and Akira, S. (1999) Immunity 10, 39–49
6. Yoo, J. Y., Huse, D. L., Nathans, D., and Desederio, S. (2002) Cell 108, 331–344
7. Nishiki, S., Hato, F., Kamata, N., Sakamoto, E., Hasegawa, T., Kimura-Rito, A., Hino, M., and Kitagawa, S. (2004) Am. J. Physiol. Cell Physiol. 286, C1302–C1311
8. Epling-Burnette, P. K., Zhong, B., Bai, F., Jiang, K., Bailey, R. D., Garcia, R., Jove, R., Djeu, J. Y., Loughran, T. P., Jr., and Wei, S. (2001) J. Immunol. 166, 7486–7495
9. Hasegawa, T., Suzuki, K., Sakamoto, C., Ohta, K., Nishiki, S., Hino, M., Tatsumi, N., and Kitagawa, S. (2003) Blood 101, 1164–1171
10. Benekli, M., Baer, M. R., Baumann, H., and Wetzler, M. (2003) Blood 101, 2840–2846
11. Schaefer, T. S., Sanders, L. K., Park, O. K., and Nathans, D. (1997) Mol. Cell. Biol. 17, 5307–5316
12. Chakraborty, A., and Tewary, D. J. (1998) J. Leukoc. Biol. 64, 675–680
13. Kawabata, K., Suzuki, M., Sugitani, M., Imaki, K., Toda, M., and Miyamoto, T. (1991) Biochem. Biophys. Res. Commun. 177, 814–820
14. Yao, A., Kitagawa, S., Motoyoshi, K., Azuma, E., Saito, M., and Takaku, F. (1992) Blood 79, 1553–1557
15. Roos, D., Voetman, A. A., and Meerhof, L. J. (1983) J. Cell Biol. 97, 368–377
16. Kjeldsen, L., Sengelov, H., and Borregaard, N. (1999) J. Immunol. Methods 232, 131–142
17. Nakajima, K., Powers, J. C., Ashe, B. M., and Zimmerman, M. (1979) J. Biol. Chem. 254, 4027–4032
18. Britz, M. L., and Lowther, D. A. (1981) Aust. J. Exp. Biol. Med. Sci. 59, 63–75
19. Sakamoto, C., Suzuki, K., Hato, F., Akahori, M., Hasegawa, T., Hino, M., and Kitagawa, S. (2003) Int. J. Hematol. 77, 69–70
20. Suzuki, K., Hino, M., Hato, F., Tatsumi, N., and Kitagawa, S. (1999) Blood 93, 341–349
21. Suzuki, K., Hasegawa, T., Sakamoto, C., Zhou, Y., Hato, F., Hino, M., Tatsumi, N., and Kitagawa, S. (2001) J. Immunol. 166, 1185–1192
22. Xia, Z., Salmer, R. R., Kunz, D. P., Baer, M. R., Kazim, L., Baumann, H., and Wetzler, M. (2001) Cancer Res. 61, 1747–1753
23. Benekli, M., Xia, Z., Donohue, K. A., Ford, L. A., Pixley, L. A., Baer, M. R., Baumann, H., and Wetzler, M. (2003) Blood 99, 252–257
24. Lane, A. A., and Ley, T. J. (2003) Cell 115, 305–318
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