Expression of Janus Kinase 3 in Human Endothelial and Other Non-lymphoid and Non-myeloid Cells*

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Members of the Janus kinase (Jak) family of protein tyrosine kinases have recently been implicated in the proximal signal transduction events of cytokine receptors. Jak3, a newly discovered member of this family, is believed to be normally limited in its expression to cells of the lymphoid and myeloid lineages. Herein we show that Jak3 is expressed in primary human vascular cells, as well as other non-lymphoid and non-myeloid cell types. Reverse transcriptase-polymerase chain reaction and Northern blot analysis revealed that Jak3 mRNA was expressed at low levels in human umbilical vein endothelial cells (HUVEC), human aortic smooth muscle cells (HASM), A549 (human lung carcinoma), and DLD-1 (human colon adenocarcinoma) cells. Higher basal levels of Jak3 mRNA were detected in HMEC-1 (human microvascular cell line) and HepG2 (human hepatocellular carcinoma) cells. Jak3 mRNA expression was induced in HUVEC, HMEC-1, and HASMC by treatment with interleukin-4, tumor necrosis factor-α, interferon-γ, and lipopolysaccharide. Jak3 protein was detectable at low levels in untreated HMEC-1, and these levels increased significantly with cytokine treatment. Furthermore, Jak3 protein was phosphorylated upon treatment of these cells with interleukin-4. This work shows that Jak3 is expressed or inducible in human vascular endothelial, vascular smooth muscle, and other non-lymphoid and non-myeloid cells, suggesting a broader role for Jak3 in the cytokine signal transduction of these cells.

Cytokines play critical roles in immunity and inflammation. Recent advances have elucidated some of the molecules involved in the signal transduction of cytokine receptors (1–4). Cytoplasmic protein tyrosine kinases of the Jak family (Jak1, Jak2, Tyk2) have emerged as critical components of the signal transduction cascade (5, 6). A new member of the Jak family of protein tyrosine kinases has been cloned and named Jak3 (7, 8). This protein differs from the other family members in that its expression is believed to be normally limited to cells of the lymphoid and myeloid lineages, such as natural killer cells, activated T lymphocytes, and activated monocytes (7, 9). Further studies, however, have shown that Jak3 is expressed abundantly in transformed hematopoietic and epithelial cell lines, as well as breast cancer tissue (10, 11). Physiologically Jak3 expression, however, has not been shown to occur in primary cells or tissues outside of the lymphoid or myeloid lineages.

Jak3 is involved in the signal transduction of the family of cytokine receptors that utilize the common chain (γc) of the IL-2r receptor (IL-2r), including IL-2r, IL-4r, IL-7r, IL-9r, and IL-15r (12). Jak3 associates with the γc chain and is activated in response to ligand binding (13, 14). A variety of non-lymphoid, non-myeloid cell types respond to IL-4, with vascular tissue, especially vascular endothelial cells, being profoundly affected (15). For example, vascular cell adhesion molecule-1 (VCAM-1), interleukin-6 (IL-6), and monocyte chemotactic protein-1 are expressed in endothelium in response to IL-4 (16–18). Given that endothelium is IL-4 responsive, that Jak3 binds the γc chain, and that Jak3 has not been reported to be expressed in vascular endothelium, we sought to determine whether endothelial cells express Jak3, and if so, whether they utilize Jak3 in IL-4 receptor signaling. Herein we provide the first evidence of primary human cells outside of the lymphoid and myeloid lineages expressing Jak3. We show that primary human endothelial and vascular smooth muscle cells, as well as a variety of non-lymphoid and non-myeloid cell lines, have detectable, albeit low, basal levels of Jak3. Jak3 levels are significantly increased in vascular endothelial and smooth muscle cells by stimulation with IL-1β, TNF-α, IFN-γ, and LPS. Finally, IL-4 treatment of the microvascular endothelial cell line HMEC-1 induces the phosphorylation of Jak3, thus suggesting a role of Jak3 in the signal transduction cascade of IL-4, and potentially other members of the γc chain receptor family, in cells outside of the myeloid and lymphoid lineages.

EXPERIMENTAL PROCEDURES

Cell Culture and Reagents—All reagents were purchased from Sigma unless otherwise noted. A549 (gift from M. Holtzman, Washington University, St. Louis, MO), DLD-1 (ATCC), HepG2 (from the Washington University Tissue Culture Center), HASMC (gift from K. Broschat, Monsanto-Searle, St. Louis, MO), and Jurkat cells (gift of S. Korsmeyer, Washington University, St. Louis, MO) were cultured in Dulbecco’s modified Eagle’s medium supplemented with 100 units/ml penicillin, 100 μg/ml streptomycin, 1 mM non-essential amino acids, 1 mM L-glutamine (Life Technologies, Inc., Gaithersburg, MD), 50 μg/ml 2-mercaptoethanol, and 10% fetal bovine serum (Hydclone Laboratories, Logan, UT). HMEC-1 cells (gift of E. W. Ades, Centers for Disease Control, Atlanta, GA, and T. J. Lawley, Emory University, Atlanta, GA) were cultured in MCDB-131 supplemented with 1 μg/ml hydrocortisone, 100 units/ml penicillin, 100 μg/ml streptomycin, 10% fetal bovine serum, and 1 ng/ml recombinant human epidermal growth factor (Life Technologies, Inc.). HUVEC (Clonetics Corporation, San Diego, CA) were cultured in modified Eagle’s medium supplemented with 100 units/ml penicillin, 100 μg/ml streptomycin, 1 mM non-essential amino acids, 1 mM L-glutamax (Life Technologies, Inc., Gaithersburg, MD), 50 μg/ml 2-mercaptoethanol, and 10% fetal bovine serum (Hydclone Laboratories, Logan, UT). HepG2 were cultured in modified Eagle’s medium supplemented with 10 ng/ml hydrocortisone, 100 units/ml penicillin, 100 μg/ml streptomycin, 10% fetal bovine serum, and 1 ng/ml recombinant human epidermal growth factor (Life Technologies, Inc.).

The abbreviations used are: IL, interleukin; VCAM-1, vascular cell adhesion molecule-1; TNF, tumor necrosis factor; IFN, interferon; LPS, lipopolysaccharide; HUVEC, human umbilical vein endothelial cells; RT/PCR, reverse transcriptase-polymerase chain reaction; kb, kilobase(s).

*This work was supported in part by a grant from Monsanto-Searle to L. F. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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cultured in MCD-B-131 supplemented with 1 μg/ml hydrocortisone, 10 μg/ml heparin, 100 units/ml penicillin, 100 μg/ml streptomycin, 5% fetal bovine serum, and 12 μg/ml bovine brain extract (Clontech). Human recombinant IL-1β, IL-4, and TNF-α were obtained from R&D Systems (Minneapolis, MN). Human recombinant IFN-γ was obtained from Biosource International (Camarillo, CA).

Detection of Jak3 in Human Endothelial and Other Non-lymphoid and Non-myeloid Cells by RT/PCR—Total RNA was prepared using the method of Chomczynski and Sacchi (19), and cDNA was prepared using Moloney murine leukemia virus reverse transcriptase (Life Technologies, Inc.). Initial detection and cloning of the 0.64-kb jk3 amplon from HUVEC cDNA was performed by PCR (Taq polymerase, Life Technologies, Inc.) using a sense primer corresponding to NcoI cloning site (5'-gagagcggccgacgctacctgcacctcagctcgcg-3'), and an antisense primer with a Clal donor site linking (5'-gagagcggccgacgctacctgcacctcagctcgcg-3'). PCR conditions were as follows: 93°C for 3 min, then 35 cycles of 93°C for 60 s, 65°C for 50 s, and 72°C for 120 s, and finally one cycle at 72°C for 3 min. The 0.64-kb jk3 amplon was digested with NotI and Clal, subcloned into the Bluescript pBSSKII vector (Stratagene, La Jolla, CA), and sequenced (Sequenase, Amer sham). The amino acid sequence was predicted from the nucleic acid sequence and was compared to published sequences, using the Gene Works sequence analysis program.

For RT/PCR detection of jk3 transcripts, cDNA was prepared from a variety of cells, and jk3 target sequence was amplified as described above. The PCR conditions were size-fractionated on a 1% agarose gel, which was then dried. The gel was then hybridized with a mixture of two primers specific for jk3 and internal to the primers used for the initial amplification. The primers were labeled with T4 polynucleotide kinase and [γ-32P]ATP. Hybridization and washing of the dried gel was done as described previously (20). The sequences of the internal primers are as follows: 5'-atggatgatgagagcggccgacgctacctgcacctcagctcgcg-3', and 5'-atggatgatgagagcggccgacgctacctgcacctcagctcgcg-3'. For the detection of β-actin by PCR, the above PCR conditions were used on parallel cDNA samples with the following β-actin specific primers: 5'-atcgatgagagccccgggctc-3' and 5'-atcgatgagatgagagccccgggctc-3'. The gel was then stained with ethidium bromide and photographed.

Northern Blot Analysis—Forty micrograms of total RNA from each sample was run on a 1% denaturing agarose gel, and blotted onto a Zeta-Probe membrane (Bio-Rad). A 3.4-kb cDNA containing the entire Jak3 kinase was obtained from HUVEC cDNA by using long Zeta-Probe membrane (Bio-Rad). A 3.4-kb cDNA containing the entire Jak3 coding region was obtained from HUVEC cDNA by using long Zeta-Probe membrane (Bio-Rad). A 3.4-kb cDNA containing the entire Jak3 coding region was obtained from HUVEC cDNA by using long Zeta-Probe membrane (Bio-Rad). A 3.4-kb cDNA containing the entire Jak3 coding region was obtained from HUVEC cDNA by using long Zeta-Probe membrane (Bio-Rad). A 3.4-kb cDNA containing the entire Jak3 coding region was obtained from HUVEC cDNA by using long Zeta-Probe membrane (Bio-Rad). A 3.4-kb cDNA containing the entire Jak3 coding region was obtained from HUVEC cDNA by using long Zeta-Probe membrane (Bio-Rad). A 3.4-kb cDNA containing the entire Jak3 coding region was obtained from HUVEC cDNA by using long Zeta-Probe membrane (Bio-Rad). A 3.4-kb cDNA containing the entire Jak3 coding region was obtained from HUVEC cDNA by using long Zeta-Probe membrane (Bio-Rad). A 3.4-kb cDNA containing the entire Jak3 coding region was obtained from HUVEC cDNA by using long Zeta-Probe membrane (Bio-Rad). A 3.4-kb cDNA containing the entire Jak3 coding region was obtained from HUVEC cDNA by using long Zeta-Probe membrane (Bio-Rad). A 3.4-kb cDNA containing the entire Jak3 coding region was obtained from HUVEC cDNA by using long Zeta-Probe membrane (Bio-Rad). A 3.4-kb cDNA containing the entire Jak3 coding region was obtained from HUVEC cDNA by using long Zeta-Probe membrane (Bio-Rad). A 3.4-kb cDNA containing the entire Jak3 coding region was obtained from HUVEC cDNA by using long Zeta-Probe membrane (Bio-Rad). A 3.4-kb cDNA containing the entire Jak3 coding region was obtained from HUVEC cDNA by using long Zeta-Probe membrane (Bio-Rad). A 3.4-kb cDNA containing the entire Jak3 coding region was obtained from HUVEC cDNA by using long Zeta-Probe membrane (Bio-Rad). A 3.4-kb cDNA containing the entire Jak3 coding region was obtained from HUVEC cDNA by using long Zeta-Probe membrane (Bio-Rad). A 3.4-kb cDNA containing the entire Jak3 coding region was obtained from HUVEC cDNA by using long Zeta-Probe membrane (Bio-Rad). A 3.4-kb cDNA containing the entire Jak3 coding region was obtained from HUVEC cDNA by using long

For phosphotyrosine immunoblots, membranes were blocked with 4% dry milk overnight at 4°C, then incubated with 1 μg/ml jk3 or jk3 antisera in Tris-buffered saline and 0.05% Tween 20 (TBS/Tween) for 1 h at room temperature. The membranes were washed with TBS/Tween 30 min, and then incubated for 30 min with a 1:750 dilution of a horseradish peroxidase-linked goat anti-rabbit antibody (U. S. Biochemical Corp.). Membranes were washed again for 30 min, and developed by enhanced chemiluminescence (ECL, Amersham).

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Detection of jk3 in Human Vascular Endothelial Cells— Initial RT/PCR screening of cDNA obtained from HUVEC with jk3 specific primers revealed a 0.64-kb amplon, as predicted by the cDNA sequence (data not shown) (7). The HUVEC amplon was subcloned, sequenced, and shown to be essentially identical to the published sequence for jk3, differing in only two places (Fig. 1). A 3-base pair deletion resulted in a loss of serine at position 112, and a single nucleotide change at position 152 replaced threonine with alanine. Further PCR screening with human jk3 specific primers showed jk3 expression in the monocyteic cell line U937, the T cell line MT2, HASMC, A549, and HMEC-1 cells (data not shown). PCR analysis using analogous murine jk3 specific primers showed jk3 expression in the murine fibroblast cell line L929, and in the murine macrophage cell line RAW 264.7 (data not shown).

Since RT/PCR screening showed the presence of jk3 mRNA, we wanted to determine if jk3 protein was detectable. HMEC-1 and Jurkat cell lysates were immunoprecipitated with jk3 and jk2 specific antisera, and immunoblotted for jk3 (Fig. 2A). A band of approximately 120 kDa was strongly detected in Jurkat cell lysates. A less intense band of similar size was detected in HMEC-1 lysates, indicating lower levels of jk3 expression relative to Jurkat cells. No jk3 specific band was seen in the jk2 immunoprecipitates from HMEC-1 cells, nor in immunoprecipitates using jk3 antisera saturated with specific peptide, thus establishing the specificity of the jk3 antisera. A faint band was noted in the jk2 immunoprecipitates from Jurkat cells that corresponded in size to jk3 protein, which may represent a slight cross-reactivity of jk2 and jk3 antisera. Western blot films were analyzed using ECL. Membranes were then stripped and reprobed with jk3 antisera, showing a strong jk2 band in the Jurkat and HMEC-1 cell lines (Fig. 2B). The jk2 band ran slightly higher than the jk3 band, as expected by their sizes. A faint band corresponding to jk3 protein was detected in Jurkat cell lysates immunoprecipitated with jk3 specific antisera saturated with jk3 specific antisera, then immuno- blotting with jk3 antisera. This is also consistent with slight cross-reactivity of the jk2 antisera.

Jk3 is induced upon cytokine treatment of vascular endothelial and vascular smooth muscle cells—Recently, the expression of jk3 has been shown to be induced in human monocyes by treatment with LPS and IFN-γ (9). Endothelium is responsive to LPS and IFN-γ, as well as to the inflammatory cytokines IL-1β and TNF-α. We therefore treated a variety of non-lymphoid and non-myeloid cell types with a cytokine mixture consisting of IL-1β, TNF-α, IFN-γ, and LPS to determine the inducibility of jk3 mRNA by RT/PCR analysis (Fig. 3A). jk3 mRNA was detected at low levels in untreated HUVEC, HASMC, A549 cells, and the human colon adenocarcinoma cell line DLD-1. Higher basal levels of jk3 mRNA were present in untreated HMEC-1, HepG2, and Jurkat cells. jk3 expression was profoundly increased in HUVEC, HMEC-1, and HASMC following an 18-h incubation of the cells with the cytokine
mixture. Treatment of A549, DLD-1, and HepG2 cells with the same mixture did not induce further expression of Jak3 mRNA. PCR controls using β-actin-specific primers confirmed that equivalent amounts of cDNA were used in all reactions (Fig. 3B). PCR reactions without added cDNA showed no Jak3 or β-actin specific bands (Fig. 3).

To further support our RT/PCR findings, we obtained a full-length human Jak3 cDNA by PCR, and used it to probe Northern blots of total RNA isolated from the same cell types (Fig. 4A). Jak3 mRNA levels were low or undetectable in unstimulated HUVEC, HMEC-1, A549, and DLD-1 cells. Unstimulated Jurkat and HepG2 cells had higher basal levels of Jak3 mRNA. Cytokine stimulation of HUVEC, HMEC-1, and

![Fig. 1. A comparison of the amino acid sequences of a 0.64-kb amplicon obtained from human endothelial cells to other published Janus kinase family members. Amino acid sequences were identical except at two positions (asterisks). Amino acid sequences that are identical across the Janus kinase family are boxed, while regions of high similarity are shaded. References for sequences are as follows: hJak3 NK lymphocyte (7), mJak3 B lymphocyte (8), mJak2 BM monocyte and hJak1 fibroblast (29), and hTyk2 T lymphocyte (30).](image1)

![Fig. 2. Jak3 protein is present in human endothelial cells. A, HMEC-1 and Jurkat cell lysates from fifty million cells were immunoprecipitated with Jak2 and Jak3 specific antisera, and then immunoblotted for Jak3. Specificity of the Jak3 antisera was confirmed in Jurkat lysates by preincubating the Jak3 antisera with specific peptide (GCETHAFTAHPEGKHLSFS) prior to Jak3 immunoprecipitation (Jak3 pep). B, the membrane in panel A was stripped and reblotted with Jak2 antisera. The Jak2 protein runs slightly higher than the Jak3 protein as predicted by their sizes.](image2)

![Fig. 3. A 0.64-kb Jak3 amplicon is detected in several non-lymphoid and non-myeloid cell types. A, Jak3 expression in HUVE, HASMC, DLD-1, A549, Jurkat, HepG2, and HMEC-1 cells. Cells were untreated (−) or treated (+) for 18 h with a cytokine mixture (cyto) consisting of IL-1β (0.5 ng/ml), TNF-α (0.5 ng/ml), LPS (10 μg/ml), and IFN-γ (100 units/ml). Jurkat cells were treated with phorbol 12-myristate 13-acetate (1 ng/ml) and calcium ionophore A23187 (1 μM) for the same time. B, β-actin expression (1.1 kb) of the same cDNA samples confirming that equivalent amounts of cDNA was used in each RT/PCR reaction.](image3)
DISCUSSION

The specificity of tissue expression of Jak3 has been a matter of controversy. Our findings of the presence of Jak3 in vascular and other non-myeloid and non-lymphoid cells is consistent with the fact that a variety of cell types respond to IL-4 (15, 21), and that the IL-4 receptor utilizes the γc chain and Jak3 (12). Also, Jak3 has been identified in human breast cancer cells and other epithelial cell lines (10, 11). However, physiologic Jak3 expression appeared to be predominately limited to lymphoid and myeloid cell types (7, 8). Herein we show that a variety of non-lymphoid, non-myeloid human cells express Jak3. More importantly we show that primary human endothelial and vascular smooth muscle cells express Jak3, and that this expression can be induced in response to inflammatory cytokines. The preliminary reports on the normal expression pattern of Jak3 were not done with cytokine-stimulated tissues (7, 8), and thus its expression in these tissues may have been missed. Furthermore, these studies only assessed Jak3 expression by Northern blot analysis. To detect significant levels of Jak3 mRNA in HMEC-1, HUVEC, and HASMC by Northern blot, it is necessary to treat cells with cytokines, as is the case in human monocytes (9). We were, however, able to detect Jak3 in a variety of cell types without stimulation by RT/PCR analysis (Fig. 3). Although our RT/PCR studies were not done in a semi-quantitative manner, the results directly paralleled the findings of Northern blot analysis (Fig. 4). This, together with the β-actin data, argue against the possibility that the higher Jak3 levels detected by RT/PCR in cytokine-treated cells resulted from varying quantity or quality of cDNA in the reactions. Furthermore, the RT/PCR analysis is useful in that it permits detection of Jak3 transcripts when Northern blot shows no apparent Jak3 transcripts. This added sensitivity is significant when assessing whether a particular cell type utilizes Jak3 for cytokine signaling, since a negative Northern blot does not rule out the possibility that Jak3 is expressed at low levels. Whether this low level of expression of Jak3 is sufficient for signaling remains to be seen. Also, the low level of expression of Jak3 in unstimulated endothelial cells also suggests why tyrosine-phosphorylated Jak3 was not detected in phosphotyrosine blots from HUVEC stimulated with IL-4 alone (22). We were able to detect the IL-4 enhancement of tyrosine phosphorylated Jak3 in HMEC-1 cells only after pretreating cells with a cytokine mixture. Our data suggest that human vascular cells, and possibly other non-myeloid and non-lymphoid cells can be induced to express significant levels of Jak3.

Vascular endothelial cells form the interface between blood and tissue, playing an important role in organ physiology and pathology. For example, expression of adhesion molecules and secretion of inflammatory proteins by endothelium is essential for the adhesion, activation, and transendothelial migration of immune cells (23). Cytokines are important regulators of endothelial cell function, with IL-4 having numerous positive and negative effects. IL-4 inhibits the production of the chemokine RANTES induced by TNF-α and IFN-γ in endothelium, and inhibits the expression of tissue factor and the down-regulation...
of thrombomodulin expression induced by TNF-α, IL-1β, and LPS in endothelium (24, 25). IL-6 and monocyte chemotactic protein-1 are expressed in endothelium in response to IL-4 (16, 17). VCAM-1 expression on endothelium is induced by IL-4, also synergizing with TNF-α in this regard (18). The function of Jak3 in these responses is unknown. Jak3 is present in unstimulated endothelium at very low levels, and it is unknown whether these levels are significant enough to allow for efficient signaling through the IL-4 receptor. It is possible that the aforementioned synergistic actions of TNF-α and IL-4 results from the TNF-α induced production of Jak3, which then allows for a greater response to IL-4. In support of this, we have shown that TNF-α is sufficient to induce the expression of Jak3 in endothelium.2 Recently, however, it was shown that the synergy between IL-4 and TNF-α in the expression of VCAM-1 on HUVEC occurs in the presence of protein synthesis inhibitors, thus arguing against the possibility that Jak3 synthesis was necessary for the synergy (18). Jak3 expression, however, was not directly assessed in this study, nor in any of the aforementioned studies. What affect the induction of Jak3 has on these responses is a topic of further investigation.

Our data indicate that vascular cells can be induced to express Jak3 in vitro. Whether this response is seen in vivo is unknown. Furthermore, what role Jak3 may be playing in vascular physiology and pathobiology is also under investigation. VCAM-1 induction by IL-4 and TNF-α, and the effects of Jak3 expression in these responses may be important to the pathogenesis of atherosclerosis. VCAM-1 binds to α4β1 and α4β7 integrins on circulating monocytes and lymphocytes (23). It has been postulated that this interaction is important for the recruitment of these cells to sites of inflammation and atherosclerotic lesions (26). We are currently determining if Jak3 is present in endothelium in atherosclerotic lesions, as well as in endothelium and other tissues during inflammatory responses.

Jak3 activation has been shown to be critical to the IL-4 induced proliferative response of the human premyeloid cell line TF-1 (27). Furthermore, endothelium has been shown to proliferate in response to IL-4 (28). Whether the induction of Jak3 in endothelium may affect its proliferative response to IL-4 remains to be determined. The ability of previously quiescent endothelium to proliferate is an essential component of the physiologic angiogenic response of wound repair, as well as the pathophysiologic angiogenic response in diseases such as rheumatoid arthritis, diabetic retinopathy, and solid tumor formation. The determination of Jak3 involvement in these responses will be of interest.

These results also show that the level of expression of Jak3 can vary in tumor cell lines (Figs. 3 and 4). Our data shows that Jak3 was present at significant levels in HepG2 cells, while it was detectable at low levels and undetectable in A549 and DLD-1 cells. These cell lines may be useful research tools in studying Jak3 signaling. Kinase-deficient cell lines have been used to study the role of other kinases in cytokine signaling.

For example, mutant cell lines were used to establish the requirement for Jak1 and Jak2 in the response to IFN-α and IFN-γ (5).

It is clear that primary human vascular cells, as well as other cells outside of the lymphoid and myeloid lineages express or can be induced to express significant levels of Jak3. The function of Jak3 in these cells, and the new functions acquired upon its induction remain to be elucidated.

Acknowledgments—We are deeply grateful to Robert Schreiber for making it possible for us to explore this area. We thank the Protein Chemistry Laboratory for primers, and Michael Holtzman, Kathy Broschat, and Stanley Korsmeyer for cell lines. Thanks also to Andy Chan, Andrey Shaw, and Emil Unanue for thoughtful comments on this manuscript.

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J. Biol. Chem. 1996, 271:13976-13980.
doi: 10.1074/jbc.271.24.13976

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