Novel Role of Janus Kinase 1 in the Regulation of Oncostatin M Receptor Surface Expression*

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The oncostatin M receptor (OSMR) is part of a heterodimeric receptor complex that mediates signal transduction of the pleiotropic cytokine OSM via a signaling pathway involving Janus kinases (Jaks) and transcription factors of the signal transducers and activators of transcription (STAT) family. Upon heterologous expression of the OSMR in several cell lines, we observed that its surface expression was significantly enhanced by coexpression of the Janus kinases Jak1, Jak2, and Tyk2 but not Jak3. Chimeric receptors consisting of the extracellular region of the interleukin-5 receptor β chain and the transmembrane and intracellular part of the OSMR were similarly up-regulated on the plasma membrane when Jak1 was coexpressed. The overall expression level of these constructs did not change significantly, but Jak1 coexpression increased the amount of endoglycosidase H-resistant, fully processed OSMR chimeras. Using mutated receptor and Jak1 constructs, we were able to demonstrate that association of Jak1 with the membrane proximal region of the receptor, but not its kinase activity, is necessary for this effect. Moreover, deletion of the OSMR box1/2 region also resulted in an improved surface expression indicating that this region may contain a signal preventing efficient receptor surface expression in the absence of associated Jak1. Finally we demonstrate that in Jak1-deficient cells, the endogenous OSMR is significantly down-regulated, an effect that can be reversed by transient expression of Jak1 in these cells.

The oncostatin M receptor (OSMR) belongs to the family of class I cytokine receptors and is one of three signal transducing receptor subunits of the interleukin (IL)-6-type cytokines. Its closest relatives are the receptor for leukemia inhibitory factor, oncostatin M (OSM), ciliary neurotrophic factor, cardiotrophin-1, and the novel neurotrophin-1/B-cell stimulatory factor-3/cardiotrophin-like cytokine (2–4). Like the other IL-6-type cytokines, OSM is involved in various systemic and local responses, such as regulation of the acute phase reaction, hematopoiesis, bone remodeling, and homeostasis of the extracellular matrix, and can act as a mediator for both the proliferation and the growth arrest of various cell lines (3, 5–7). In contrast to all other members of this family, which transduce their signals either via a gp130 homodimer or a gp130/leukemia inhibitory factor receptor heterodimer, OSM has the unique property to signal via a receptor complex comprising gp130 and the OSMR (1, 3).

As the signal transducing receptor chains of IL-6-type cytokines do not contain an intrinsic kinase activity, a property shared with most other cytokine receptors, signaling is mediated by the Janus family of tyrosine kinases. These cytokoplasmic kinases contain a kinase and a kinase-like domain in their C-terminal region, whereas their N-terminal region mediates constitutive association with cytokine receptors (8). On the basis of sequence similarities, it has been proposed that this region contains a FERM domain (as present in four-point-one, ezrin, radixin, and moesin). A recent mutagenesis study based on a molecular model has highlighted the importance of the N-terminal subdomain F1 with a β-grasp fold for the interaction with gp130 (9).

Within the cytokine receptors, the membrane proximal box1/box2 region has been demonstrated to be required for Jak binding. This region is characterized by a proline-rich motif (box1) and a less conserved region dominated by a hydrophobic stretch of amino acids followed by several charged amino acids (box2) (3). Although activation of the Janus kinases Jak1, Jak2, and Tyk2 has been reported for IL-6-type cytokines (3, 10), studies carried out in Jak1-deficient cells have established that Jak1 plays the dominant role in signal transduction of this family of cytokines (11, 12).

Upon ligand binding and receptor dimerization, Jaks are activated and in turn phosphorylate several cytoplasmic tyrosine residues in the gp130-OSMR complex that then provide docking sites for SH2 domain-containing molecules, such as the transcription factors STAT3 and -1, the negative feedback inhibitor suppressor of cytokine signaling 3, and the tyrosine phosphatase SHP-2 or the adaptor protein Shc, which may each contribute to activation of the mitogen-activated protein kinase pathway (3, 10, 13–16).

It has therefore been widely accepted that Jaks play an important role in cytokine signaling, and only cytokine receptors that are able to bind Jaks are signaling-competent (3). However, not much is known about how functional receptor-Jak complexes are generated. Interestingly studies

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† The abbreviations used are: OSMR, oncostatin M receptor; OSM, oncostatin M; Endo H, endoglycosidase H; STAT, signal transducers and activators of transcription; Jak, Janus kinase; IL, interleukin; gp, glycoprotein; EPO/R, erythropoietin receptor; ER, endoplasmic reticulum; HLA, human histocompatibility leukocyte antigen; PBS, phosphate-buffered saline; FACS, fluorescence-activated cell sorting; CFP, cyan fluorescent protein.

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carried out with the erythropoietin receptor (EPOR) demonstrated that Jak2/EPOR association takes place very early in biosynthesis, namely in the ER (17).

In this article we demonstrate that the expression of Jak1 promotes the surface expression of the OSMR. This effect is mediated by association of Jak1 with the receptor, whereas Jak1 kinase activity is not required. We further show that the OSMR contains a signal within its box1/2 region that prevents efficient surface expression and that may be masked upon Jak binding to the OSMR. We propose that such a mechanism serves as a quality control, ensuring that only functional receptor-kinase complexes are expressed at the cell membrane.

MATERIALS AND METHODS

Cell Culture and Transfection—COS-7 simian monkey kidney cells, 3T3 murine fibroblasts, HeLa human cervix carcinoma cells, and human 293T, U4A, and U4C fibrosarcoma cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% (v/v) fetal-calf serum, 100 µg/ml streptomycin, and 50 µg/ml penicillin. Cells were grown at 37 °C in a water-saturated atmosphere in air/CO2 (19:1). Cells were transiently transfected using DEAE-dextran (COS-7) as described previously (15, 18). FuGENE transfection reagent (COS-7 and 3T3), or Superfect transfection reagent (HeLa, U4A, and U4C) was obtained from the manufacturer (Roche/Boehringer and Qiagen). For receptor expression in COS-7 cells, the pSVL expression vectors (Amersham Biosciences Inc.) were used, whereas HeLa and 3T3 cells were transfected with pCAGGS expression vectors (19).

Expression Constructs—The expression plasmid containing the human OSMR cDNA was a generous gift of Dr. B. Mosley (Immunex). The plasmid was digested with XhoII and BglII, and the insert containing the OSMR cDNA was cloned into XhoII/BamHI-digested pSVL or into XhoII/BglII-digested pCAGGS vector. The construction of the IL-5R-Δchimeras /βOSMR, β/OSMR1, and β/OSMR-box1/2 has been described previously (15, 18). The C-terminal deletion mutants β/OSMR-box1 and β/OSMRαcty were generated by PCR using antisense oligonucleotides incorporating an in-frame termination codon followed by the recognition sites for BamHI. They retain 18 and 3 amino acids, respectively, of the OSMR cytoplasmic tail. The construct β/OSMR1-mutbox1 containing the amino acid substitutions P775A and P777A and the deletion construct β/OSMR1-Δbox1 were generated by PCR using the respective oligonucleotides with the cDNA for β/OSMR1 as a template. The resulting products were cloned into the EcoRI/BamHI-digested expression vector pSVL or pCAGGS/OSMR.

The generation of Jak1 constructs L80A/Y81A and Y107A and of the IL-5R Δchimeras /βOSMR, IL-5RΔbox1 were generated by PCR using the respective primers, with the cDNA for OSMR-box1 as a template. The resulting products were cloned into the EcoRI/BamHI-digested expression vector pSVL or pCAGGS/OSMR.

Antibodies—The monoclonal α-STAT3 antibody was obtained from Transduction Laboratories (Lexington, KY). Monoclonal α-IL-5Rβ (S-16), polyclonal α-IL-5Rβ (N-20), polyclonal α-Jak3 (C-21), polyclonal α-Tec (M-20), and polyclonal α-OSMR (AN-A2) antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Monoclonal α-HLA class I antibody was purchased from Sigma, and horseradish peroxidase-, tetramethylrhodamine B isothiocyanate-, and fluorescein isothiocyanate-conjugated secondary antibodies were obtained from Dako (Hamburg, Germany). R-phycocerythrin-conjugated α-mouse IgG Fab fragments were from Dianova (Hamburg, Germany). The polyclonal antiserum against Jak1 was a kind gift from Dr. A. Ziemiecki (Bern, Switzerland).

Flow Cytometry—5 × 10⁵–1 × 10⁶ cells were resuspended in cold PBS supplemented with 5% fetal calf serum and 0.1% sodium azide (PBS/azide) and incubated with 1 µg/ml of α-OSMR or α-IL-5Rβ antibody (S-16) for 30 min at 4 °C. Cells were washed with cold PBS/azide and incubated in darkness with a 1:50 dilution of an R-phycocerythrin-conjugated α-mouse antibody for 30 min at 4 °C. Cells were washed again and resuspended in PBS/azide. 10⁵ cells/sample were analyzed by flow cytometry using a FACScalibur (Becton Dickinson) equipped with a 488 nm argon laser.

Cell Lysis, Immunoprecipitation, Endo Digestion, and Western Blot Analysis—Cells were lysed in 500 µl of lysis buffer (20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 10 mM NaF, 1 mM sodium vanadate, 10 mM phenylmethylsulfonyl fluoride, 1 mM benzamidine, 5 µg/ml aprotinin, 3 µg/ml pepstatin, 5 µg/ml leupeptin, 1 mM EDTA, and 1% Triton X-100) or 1% Brij97 (for coinmunoprecipitations and Endo H treatment). Receptors were immunoprecipitated from cleared lysates using α-IL-5Rβ (S-16) antibody. The immune complexes were collected on protein A-Sepharose and washed twice with washing buffer (lysis buffer with only 0.1% Brij97). For Endo H digestions, the immunoprecipitates were washed once in 200 µl sodium citrate (pH 5.5) and incubated in 100 µl of citrate buffer for 18–24 h with or without 5 µmliters of Endo H. Subsequently the Sepharose was boiled for 5 min in Laemmli buffer at 95 °C. Proteins were separated by SDS-PAGE in 7.5% gels (lysates and coinmunoprecipitates) or 10% gels (Endo H analysis) in a buffer containing 1% sodium dodecyl sulfate. After SDS-PAGE, gels were stained with Coomassie blue or transferred onto a polyvinylidene difluoride membrane (PALL Biotechnology, Germany). Western blot analysis was conducted using the indicated antibodies and the enhanced chemiluminescence kit (Amersham Biosciences, Inc.) according to the instructions of the manufacturer. Before reprobing, blots were stripped in 2% SDS, 100 mM β-mercaptoethanol in 62.5 mM Tris-HCl (pH 6.7) for 20 min at 75 °C.

Immunofluorescence Microscopy—Transfected cells growing on coverslips were fixed in 2% paraformaldehyde and treated for 5 min with 50 mM NH4Cl. Cells were blocked for 30 min in PBS containing 1 mM MgCl2, 0.1 mM CaCl2 (PBS + ++), and 1% bovine serum albumin and incubated for 30 min with primary antibody (diluted 1:100 in PBS +++, 0.2% bovine serum albumin). After washing the cells three times with PBS +++, 0.2% bovine serum albumin, cells were labeled with secondary antibody diluted 1:200 in PBS +++, 0.2% bovine serum albumin. Cells were washed again, mounted in Moviol, and analyzed on the next day with a Zeiss confocal laser scanning microscope (LSM 510). For staining of intracellular receptors, cells were permeabilized using solutions additionally containing 0.1% Triton X-100.

RESULTS

Surface Expression of Oncostatin M Receptor Is Up-regulated by Coexpression of Jak1—COS-7 cells were transiently transfected with expression plasmids for the OSMR and either the Janus kinase Jak1 or the cytoplasmic tyrosine kinase Tec as a control. When we monitored the cellular localization of the OSMR by immunofluorescence studies, we became aware of a novel function of Jak1. While COS-7 cells expressing the OSMR and Tec kinase generally displayed a moderate surface staining of the OSMR (Fig. 1A, first panel), coexpression of OSMR and Jak1 resulted in an increased number of cells with a bright surface staining (Fig. 1A, third panel). However, in both cases, a large portion of the OSMR was located intracellularly (Fig. 1A, second and fourth panels). By flow cytometry, we were able to verify the immunofluorescence data. A much higher number of cells coexpressing Jak1 display a strong OSMR surface staining compared with cells coexpressing Tec (Fig. 1B, compare upper and lower histogram). Furthermore, the mean fluorescence of transfected cells, which was calculated by histogram subtraction, was strongly increased in Jak1-coexpressing compared with Tec-coexpressing cells (Fig. 1B, right panel). To exclude that coexpression of Tec kinase might have a negative effect on OSMR expression, we also transfected the empty vector or an expression vector for STAT1 as controls. In both cases, similar results were obtained (data not shown). Furthermore, the up-regulation of OSMR surface expression by coexpression of Jak1 was not restricted to COS-7 cells as we observed the same effect in transfected human HeLa cervix carcinoma and murine 3T3 fibroblasts cells (Fig. 1C).

Coexpression of Jak1 Alters the Cellular Localization and the Glycosylation State of Heterologously Expressed OSMR Chimeras—The Jak-mediated up-regulation of OSMR surface expression could be due to an increased overall expression of heterologously expressed OSMR. Therefore, it was important to monitor whether Jak overexpression results in an altered expression level of the OSMR. Since antibodies recognizing the OSMR in Western blot analysis are currently not available, we took advantage of receptor chimeras consisting of the extracel-
lular region of the IL-5Rβ fused to the OSMR transmembrane and intracellular region (Fig. 2A). These are readily detectable in Western blot analysis and flow cytometry as shown previously (15, 18).

We first tested whether the results obtained with the OSMR could be confirmed with the IL-5Rβ/OSMR constructs. When we carried out immunofluorescence studies using the deleted chimera β/OSMRΔ1, which is generally better expressed than the full-length construct (18), almost no surface staining was detectable in cells expressing this chimera without Jak1 (Fig. 2B, first panel), whereas we obtained bright surface staining when coexpressing Jak1 (Fig. 2B, third panel). Similar to the pattern observed for wild type OSMR (Fig. 1A), a large portion of the heterologously expressed β/OSMRΔ1 chimeras was present in intracellular compartments (Fig. 2B, second and fourth panels). Again we carried out FACS analysis to further assess the surface expression of chimeric receptor proteins. In COS-7 cells coexpressing Jak1, the surface expression of both β/OSMR and β/OSMRΔ1 was up-regulated (Fig. 2C, upper panel). It should be noted that in immunofluorescence staining as well as
Jak1 Up-regulates OSMR Surface Expression

**FIG. 2.** Coexpression of Jak1 alters the cellular localization and the glycosylation pattern but not the overall protein level of transiently expressed OSMR chimeras. COS-7 cells were transiently transfected with the expression vectors for chimeric receptors as indicated and for the kinase Jak1 or Tec as a control (co). Cells were harvested the next day. **A,** schematic representation of constructs used in this study. The extracellular parts are represented in dark gray (OSMR) or light gray (IL-5Rβ). White boxes in the intracellular region represent box1 and box2, the black box represents a mutated box1, and black lines indicate tyrosine residues. Details about the mutations are indicated on the right side of the figure. **B,** immunofluorescence staining of transiently transfected COS-7 cells. Staining was performed as explained in the legend to Fig. 1A with the exception that an α-IL-5Rβ antibody was used. **C** FACS and Western blot analysis of transfected COS-7 cells. FACS analysis was carried out using monoclonal α-IL-5Rβ and secondary phycoerythrin-conjugated antibodies. The relative surface expression was calculated as described in the legend to Fig. 1B. Mean values and S.D. of at least three independent experiments are depicted. For Western blot analysis, cells were lysed in Triton lysis buffer, and equal amounts of lysates were separated by 7.5% SDS-PAGE. The blot was stained with anti-serum against IL-5Rβ. **D,** Endo H analysis of transfected receptors. Cells were lysed in Brij lysis buffer, and receptors were immunoprecipitated using monoclonal α-IL-5Rβ antibody. Samples were divided into two aliquots and incubated overnight with or without 5 milli-units of Endo H. Precipitates were separated by 10% SDS-PAGE, and Western blots were stained with polyclonal anti-sense antisera against IL-5Rβ. Endo H-resistant receptor bands are marked by a closed arrow. Open arrows mark Endo H-sensitive receptor forms. **IP,** immunoprecipitation; **D,** detection.

In FACS analysis (data not shown), the signals obtained by staining the IL-5Rβ chimeras were weaker compared with signals obtained from staining the OSMR, probably due to different affinities of the antibodies used. However, the relative up-regulation of the β/OSMR as measured by FACS analysis was comparable to that observed for the OSMR (Fig. 1B). Importantly the steady state receptor levels of β/OSMR did not change significantly upon Jak1 coexpression as demonstrated by Western blot analysis (Fig. 2C, lower panel). However, Endo H analysis of immunoprecipitated chimeras revealed that the glycosylation state of receptors was altered in cells coexpressing Jak1. When we expressed β/OSMRΔ1 without Jak1, most of the precipitated receptors were Endo H-sensitive (Fig. 2D, lanes 1 and 2) indicating that these receptors had not been fully processed yet and still contain N-glycans of the high mannose type susceptible to Endo H digestion. Upon Jak1 coexpression, an additional Endo H-resistant protein band of lower mobility was detectable (Fig. 2D, lanes 3 and 4, filled arrowheads) representing the fully processed receptor form carrying complex-type N-glycans that cannot be removed by Endo H. (It should be noted that detection of the two different forms of β/OSMRΔ1 depends on a good resolution in the SDS gel. Under other conditions, it is hardly possible to discriminate these two bands.) However, a large portion of the immunoprecipitated receptors were still Endo H-sensitive (Fig. 2D, lanes 3 and 4, open arrowheads). These results thus match the data achieved from immunofluorescence analysis.

**Jak1 Binding to the Receptor, but Not Its Kinase Activity, Mediates the Increased Surface Expression of the Chimeric OSMR**—We next examined whether binding of Jak1 to the receptor is needed for OSMR up-regulation at the plasma membrane. Since it is known that the proline-rich box1 region of cytokine receptors is important for Jak recruitment (see Ref. 3), we generated chimeric receptors in which the two proline residues, 775 and 777, of box1 were exchanged by a glutamic acid (box1mut) (Fig. 2A). These results thus match the data obtained from the binding analysis.

**Fig. 3** Jak1 binding to the receptor.
We have recently shown that two specific Jak1 mutants with amino acid exchanges in their N-terminal β-grasp domain, Jak1 L80A/Y81A and Jak1 Y107A, completely lost their ability to bind to gp130 (9). When we coexpressed the chimera β/OSMRΔ with these mutants or wild type Jak1, only the latter led to receptor up-regulation (Fig. 3B, upper panel). Neither mutant bound to the OSMR chimera even though both were expressed to a similar extent as wild type Jak1, which readily coprecipitated with β/OSMRΔ (Fig. 3B, lower panels). This again demonstrates that Jak1 binding to the OSMR is required for receptor up-regulation at the cell surface. As we have shown in earlier studies that overexpression of Jak1 in COS-7 cells can lead to ligand-independent phosphorylation of coexpressed receptors (15), we examined next whether Jak1 kinase activity is involved in the surface up-regulation of the OSMR. We therefore coexpressed the constructs β/OSMRΔ and Jak1 K907E, which is kinase-negative due to an amino acid exchange in its kinase domain (20). Kinase activity was monitored indirectly by coimmunoprecipitation of STAT3 with the chimeric receptor (which is possible due to ligand-independent receptor phosphorylation when Jak1 is overexpressed (15)). While binding to β/OSMRΔ could be demonstrated for both Jak1 K907E and wild type Jak1, STAT3 coprecipitation was only observed when wild type Jak1 was coexpressed (Fig. 3C, middle panels). Interestingly coexpression of both wild type and the kinase-negative Jak1 with the OSMR chimera resulted in an increased surface expression of β/OSMRΔ (Fig. 3C, upper panel). Thus, the association of Jak1 with the OSMR, but not Jak1 kinase activity, is required for the up-regulation of heterologously expressed OSMR constructs.

The Membrane Proximal Region of the OSMR Contains a Signal That Negatively Regulates Receptor Surface Expression—The observed up-regulation of OSMR surface expression by coexpression of Jak1 could be due to the masking of a negative signal present in the OSMR intracellular region. The deletion of this signal should then have the same effect as its masking by binding of Jak1, namely an up-regulation of receptor surface expression. Alternatively Jaks could provide a positive signal to receptor processing. Since Jak1 binding takes place in the box1/2 receptor region, we assumed that any negative signal masked by Jak1 should also be located in this area. A detailed schematic representation of the OSMR box1/2 region is given in Fig. 4A. We examined the surface expression of OSMR chimeras still containing or lacking this region.
mutants /\textit{H9252}/OSMR-box1 and /\textit{H9252}/OSMR-box1/2 displayed similar low surface expression levels as determined by FACS analysis (Fig. 4B, upper panel). Western blot analysis and Endo H treatment revealed that these receptors were expressed mainly in the Endo H-sensitive, incompletely processed form (Fig. 4, B, lower panel, and C).

Strikingly the deletion mutants /\textit{H9252}/OSMR-box1 and /\textit{H9252}/OSMR-acyt were expressed much better at the cell surface as determined by FACS analysis (Fig. 4B, upper panel). Interestingly two different protein bands were detectable for these chimeras when monitoring receptor expression by Western blot analysis (Fig. 4B, lower panel), the upper band representing the fully processed form of these receptor constructs as analyzed by Endo H treatment (Fig. 4C). These results are therefore consistent with the presence of a negative regulatory signal in this region.
Jak1 Regulates Surface Expression of Endogenous OSMR in Human Fibrosarcoma Cells—All our data are consistent with a mechanism where Jak1 enhances OSMR surface expression by masking of a negative regulatory signal within the cytoplasmic domain of the OSMR. If this novel function of Jak1 is of relevance, Jak1 expression should also have an effect on the expression of the endogenous OSMR. We therefore compared the surface expression of the endogenous OSMR in two independently derived human fibrosarcoma cell lines lacking Jak1 (U4A and U4C) with the OSMR surface expression in their respective parental cell lines still expressing Jak1 (2ftgh and 2C4). As a control we also determined in each case the surface expression of the HLA class I molecule. While FACS analysis revealed that the HLA surface expression was independent of Jak1 expression (Fig. 5C, left panel), the OSMR surface expression was significantly reduced in U4A compared with 2ftgh cells (Fig. 5A, left panel) and in U4C compared with 2C4 cells (Fig. 5A, right panel). Transient expression of Jak1 could in each case restore the OSMR surface expression (Fig. 5B). We thus conclude that Jak1 has a positive up-regulatory effect on surface expression of endogenous receptors as well. Since transient expression of the kinase-negative mutant Jak1 KE had the same effect on OSMR surface expression as wild type Jak1 (Fig. 5B), this process does not depend on signaling events mediated by Jak1 kinase activity (which might, for example, result in transcriptional up-regulation of the OSMR).

Although OSMR surface expression was markedly reduced in U4A and U4C cells, Jak1 deficiency obviously did not lead to total loss of OSMR surface expression. However, the Janus kinases Jak2 and Tyk2 are still expressed in these cells (11, 12). Both kinases have been reported to become activated after OSM stimulation (10, 21), and Jak2 has been shown to coprecipitate with the cytoplasmic region of the OSMR (18). We therefore examined whether these kinases are able to mediate OSMR surface up-regulation as well. As seen in Fig. 5C, this is indeed the case. In COS-7 cells, coexpression of OSMR with Jak1, Jak2, or Tyk2 led in each case to a strong increase of OSMR surface expression (Fig. 5C, left panel). Importantly coexpression of Jak3, the last member of the Janus kinase family, did not alter the surface expression of the OSMR in COS-7 cells (Fig. 5C, right panel). Thus, it is well conceivable that all of the three Janus kinases involved in OSM signaling also contribute to OSMR surface expression.

FIG. 5. The surface expression of endogenous OSMR is regulated by Jak1. A, human fibrosarcoma cells deficient for Jak1 (U4A and U4C) or parental cells (2ftgh and 2C4) were examined by FACS analysis for surface expression of the OSMR and the HLA class I molecule as a control. Receptor surface expression of parental cells was set to 100%. Mean values and S.D. of at least three different experiments are depicted. B, Jak1-deficient U4A and U4C cells were transfected transiently with an expression vector for Jak1 or with empty vector (co); U4C cells were additionally transfected with DNA for the kinase-negative Jak1 KE mutant. OSMR surface expression was measured by FACS analysis as described before. OSMR surface expression in control transfected cells was set to 100%. Mean values and S.D. of at least three independent experiments are depicted. C, COS-7 cells were transiently cotransfected with expression vectors for OSMR and a Janus kinase as indicated. Surface expression was measured by FACS analysis, and receptor expression of cells cotransfected with empty vector or Tec expression plasmid was set to 100%. Mean values and S.D. of at least three different experiments are represented. For Western blot analysis, cells were lysed in Triton lysis buffer, and equal amounts of lysates were separated by 7.5% SDS-PAGE.

The Western blot was stained with polyclonal antisera against Jak1, Tec, and Jak3 as indicated. hu, human; IP, immunoprecipitation; D, detection.
DISCUSSION
In this article we provide evidence for a novel role of Jak1 in regulating the cell surface expression of the human oncostatin M receptor. We have demonstrated that Jak1 coexpression leads to an increased membrane expression of the wild type OSMR and of receptor chimeras containing the transmembrane and intracellular region of the OSMR. In contrast to that, the coexpression of the Janus kinase-unrelated tyrosine kinase Tec did not have any effect on OSMR surface expression, so we used coexpression of this kinase as a control. Similar findings were generated in various cell lines, and using two independently generated Jak1-deficient fibrosarcoma cells (U4A and U4C), we could also confirm a role of Jak1 for the expression of the endogenous human OSMR. It should be noted that OSMR surface expression was not completely lost in U4A and U4C cells. Since Jak2 or Tyk2, but not Jak3, coexpression in COS-7 cells also led to an increased OSMR surface expression, these two Janus kinases may partly replace Jak1 in promoting expression of the OSMR at the membrane. These findings are consistent with reports of Jak2 and Tyk2 activation upon stimulation with OSM (10, 21) and thus further imply a role of these kinases in OSM signaling.

The experiments presented in this study strongly support a model in which the observed OSMR up-regulation by Jak1 is due to masking of a signal within the membrane proximal region of the OSMR that prevents efficient surface expression. First, we could demonstrate that receptors that no longer bind Jak1 due to mutation or deletion of their box1 region are not up-regulated on the cell surface by Jak1 coexpression. The same result was generated when we coexpressed Jak1 constructs that had lost the ability to bind to the receptor due to mutation within their N-terminal β-grasp domain. Thus the observed OSMR up-regulation is not an unspecific effect caused by Jak1 overexpression, but Jak1-OSMR interaction is strictly required. However, Jak1 kinase activity was not needed for receptor up-regulation, so that signaling events mediated by Jak1 seem not to play a role.

Most importantly truncation of the membrane proximal receptor region of the OSMR resulted in receptors that were efficiently expressed at the membrane without coexpression of Jak1. Thus, partial deletion of the Jak1 binding region of the OSMR and coexpression of Jak1 have the same effect on the expression pattern of chimeric OSM receptors. In both cases, receptor up-regulation at the cell surface was paralleled by the increased appearance of fully processed receptor forms as seen after Endo H treatment of precipitated receptors. This finding precludes that Jaks enhance receptor surface expression by providing a positive signal, such as an ER export signal. Instead we propose that the OSMR contains a negative signal in its membrane proximal region that can be masked by associated Jaks. The fact that the membrane proximal region of the OSMR is also the region of Jak-receptor interaction is consistent with this model.

The regulation of surface expression by masking of negative regulatory signals has been mainly described for transmembrane proteins that are part of multimeric complexes such as the T-cell receptor. This receptor complex consisting of eight subunits is only expressed at the cell surface when all receptor chains are correctly assembled (22). Expression of single subunits leads to intracellular retention often followed by degradation (22, 23). In various T-cell receptor chains determinants for degradation, ER retention, and/or lysosomal targeting have been identified (24–31). Importantly all these signals can be masked by correct assembly with the other T-cell receptor chains (27, 32–34). Similar masking mechanisms have been described for the IgE receptor chain (35), for various ion channels such as the ATP-sensitive K+ channel (36) and the high voltage-activated Ca2+ channel (37), and lately for the γ-aminobutyric acid type B receptor GB1, a member of the G-protein-coupled receptor family (38). In all these cases, regulation of cell surface expression was due to masking of an ER retention and/or a lysosomal targeting signal by correctly assembled subunits. Our results indicate a similar mechanism for regulation of the OSMR surface expression. Masking of an ER retention and/or lysosomal targeting signal present in the box1/2 region by Jak1 would explain the increased portion of fully processed, surface-located receptors upon Jak1 expression. In immunofluorescence studies, chimeric receptors still containing the whole box1/2 region were predominantly found in the ER and Golgi, while receptors devoid of this region were mainly detected in the Golgi and at the membrane. Both an ER retention and a lysosomal targeting signal could result in this pattern of expression as receptors transported to the Golgi would either be targeted for degradation (lysosomal targeting) or be transported back to the ER (ER retention/retrieval mechanism). Strikingly the interbox1/2 region of the OSMR contains a dileucine-like signal matching the consensus sequence for internalization/lysosomal targeting (D/E)XXX(L/I)M as present in the CD3γ chain (32, 39), whereas none of the established ER retention/retrieval motifs, such as a C-terminal dileucine motif or arginine-containing motifs (36, 40–42), is present in the OSMR. However, sequences unrelated to these well-defined motifs may also mediate ER retention (30, 31). Further studies are required to dissect which of these mechanisms play a role in regulation of the OSMR surface expression and to identify the exact signals involved.

The expression pattern of another cytokine receptor, the EPOR, displays some similarity to the OSMR. Most of the receptors are located intracellularly and are incompletely processed. In this case, the inefficient folding of the extracellular region of the EPOR leads to intracellular retention so that a cytoplasmically deleted EPOR is also retained (43, 44). It was recently demonstrated that the membrane proximal box1/2 region of the EPOR contains information targeting this receptor for proteasomal degradation; however, a possible regulatory function of Jak2 in this process has not yet been described (45).

Interestingly Janus kinases may play a similar role for expression of the interferon α receptor chains, which belong to the class II cytokine receptor family (46, 47). For example, the steady state expression level of endogenous IFNAR1 was greatly reduced in a Tyk2-lacking cell line, an effect that could be reversed by Tyk2 expression. Similar to the results presented here, Tyk2 kinase activity was not needed to sustain IFNAR1 expression levels, but the N-terminal region of Tyk2, which mediates binding to the receptor, was important (46). Furthermore, it was shown that the surface expression of endogenous IFNAR1 is reduced upon overexpression of chimeric receptor constructs that recruit Tyk2, possibly due to sequestration of endogenous Tyk2 by the overexpressed receptors so that it is no longer able to sustain IFNAR1 expression (48). However, so far no significant differences of IFNAR1 expression levels were observed in tissues from Tyk2<sup>−/−</sup> mice compared with tissues of corresponding wild type mice perhaps due to species-specific differences (49, 50). In case of the Jak1<sup>−/−</sup> mice, our preliminary data provide evidence that Jak1 might also play a role for the expression of the murine OSMR. However, the human and the mouse OSMR differ in many aspects, and more experiments are under way to clarify whether this down-regulation is due to a mechanism similar to those discussed here for the human receptor.

Taken together our data demonstrate that OSMR surface expression is tightly controlled, and mechanisms exist to en-
sure the correct assembly of functional OSMR-Jak1 complexes. ER retention and/or lysosomal degradation of OSMRs not equipped with a Janus kinase would serve as a quality checkpoint in receptor processing and guarantee that only receptors capable of signaling reach the plasma membrane. These findings therefore contribute to our understanding of how functional cytokine receptor-kinase complexes are generated within the cell.

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similar findings for the EPOR and Jak2 were published by Huang, L. J.,...