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Depletion of Cellular Cholesterol and Lipid Rafts Increases Shedding of CD30

Bastian von Treschow,† Karl-Josef Kallen,‡ Elke Pogge von Strandmann,* Peter Borchmann,* Hans Lange,† Andreas Engert,* and Hinrich P. Hansen‡

CD30, a lymphoid activation marker, is shed into the cell environment after endoproteolytic cleavage of its ectodomain. Soluble (s)CD30 is able to suppress the Th1-type immune response. Because high serum levels of sCD30 and cholesterol-lowering drugs seem to be beneficial in some Th1-type autoimmune diseases, we focused on a link between CD30 shedding and the amount of cellular cholesterol. Cholesterol depletion of human Hodgkin lymphoma- and non-Hodgkin lymphoma-derived cell lines by methyl-β-cyclodextrin led to a down-regulation of membrane-bound CD30 and increased release of sCD30. Additionally, the cholesterol-interfering drugs lovastatin, cholesterol oxidase, and filipin increased CD30 shedding. Both the down-regulation of membrane-anchored CD30 and the release of sCD30 were dependent on metalloproteinases. Using specific inhibitors, we detected TNF-α converting enzyme (TACE) as the leading enzyme responsible for cholesterol-dependent CD30 shedding. A Triton X-100-based method for lipid raft isolation revealed that CD30 was partially present in lipid rafts, whereas TACE was localized in the nonraft fractions. Disintegration of lipid rafts by cholesterol depletion might therefore lead to dynamic interactions of CD30 with TACE, resulting in enhanced shedding of CD30. Our results suggest a possible role of cholesterol-dependent shedding of CD30 in the pathogenesis of immune diseases. The Journal of Immunology, 2004, 172: 4324–4331.

A 120-kDa type I transmembrane glycoprotein, CD30 is normally found on a small subset of lymphocytes, especially Th2 cells (1). Due to its typical cysteine-rich domains, CD30 was classified as a member of the TNFR superfamily (2). This family includes receptors like the TNFR1 and -2 (CD120a/b), CD95/Fas, CD40, and CD27, collectively involved in a wide spectrum of cellular responses, such as induction of gene expression or proliferation but also initiation of apoptosis (3).

Due to its overexpression on the malignant cells of Hodgkin’s disease (HD)† and anaplastic large cell lymphoma (ALCL), CD30 is currently evaluated as a target for the immunotherapy of certain cancers (4). In this respect, it is a severe obstacle that the CD30+ cells release a 90-kDa soluble form of CD30 (sCD30) (5), which serves as a serum competitor. The sCD30 concentrations in the serum of patients suffering from HD and ALCL are ~20 and 1500 times higher, respectively, as compared with healthy donors (6). Increased serum levels were also detected in infectious and allergic conditions such as EBV-induced mononucleosis, hepatitis virus B infection, and immunodeficiency virus type 1 infection. Some autoimmune diseases, such as systemic lupus erythematosus, rheumatoid arthritis, systemic sclerosis, atopic dermatitis, Wegner’s granulomatosis, Graves’ disease, and Hashimoto’s thyroiditis also show increased serum levels of sCD30 (7).

There are inconsistent data on the contribution of CD30+ cells and sCD30 in inflammatory processes. On the one hand, CD30 expression and its release seems to correlate with the extent of inflammation in diseases such as atopic dermatitis (8), systemic lupus erythematosus (9), and Wegner’s granulomatosis (10). In contrast, there is evidence for a counterregulatory role of CD30+ T cells at the site of inflammation in the Th1-mediated rheumatoid arthritis (11). Recently, it has been shown that this effect is also caused by sCD30, because a viral sCD30 homolog (vCD30) is able to inhibit the Th1-, but not Th2-type inflammation in vivo (12). In line with this finding, elevated serum levels of sCD30 have been found in multiple sclerosis patients in remission (13) and correlated negatively with inflammatory markers in rheumatoid arthritis (14). Because both diseases are regarded as Th1 mediated (15, 16), the elevated sCD30 levels might be beneficial in readjusting the Th1/Th2 balance.

The mechanisms that regulate sCD30 generation are poorly understood. Like many other substrates, the shedding of CD30 is catalyzed by TNF-α converting enzyme (TACE) (17). Recently, lipid rafts were found to be critical in the shedding regulation of some membrane proteins (18–20). Rafts are microdomains of the membrane that are enriched in cholesterol and sphingolipids (21). They are supposed to form ordered domains that float in the liquid-disordered matrix of the cellular membrane and play an important role in signal transduction, cell-cell interaction, and endocytosis of certain proteins. Because cholesterol-depleting agents such as methyl-β-cyclodextrin (MβCD) lead to a disintegration of lipid rafts, these agents play a role in investigating the microdomains. Besides other effects, the cholesterol-lowering drugs induce metalloproteinase-dependent shedding in some cases (18, 22). In this respect, the localization of Alzheimer’s amyloid precursor protein (APP) inside or outside of rafts seems to be decisive in terms of whether amyloidogenic or nonamyloidogenic APP is generated.
(18, 19). Moreover, the shedding regulation of the IL-6R, L-selectin, and the L1 adhesion molecule was shown to be connected to cellular cholesterol and lipid rafts, but so far, the exact mechanisms remain unclear (20, 23, 24).

The aim of this study was to examine how the cellular cholesterol content and lipid rafts influence the shedding of CD30. Our results shed light on a novel mechanism of the generation of sCD30 and provide a possible link between cholesterol and CD30-based immune regulation.

Materials and Methods

Cell culture and reagents

The mAbs Ki-1, Ki-2, Ki-3, and Ki-4 (25) were used for the detection of CD30 in flow cytometry, ELISA, immunoprecipitation, and Western blot. Sodium orthovanadate, Triton X-100, MβCD, filipin, BSA, lovastatin, peroxidase-labeled cholera toxin subunit B, HRP, FITC, isocyanate I-celte, protein A-agarose beads, cholesterol, and cholesterol oxidase were purchased from Sigma-Aldrich (Taufkirchen, Germany). Complete protease inhibitor tablets were purchased from Roche Diagnostics (Mannheim, Germany). The anti-TACE mAb MAB9302, the recombimant human TACE ectodomain Western blotting standard, and tissue inhibitor of metalloproteinases (TIMP)-1 and TIMP-3 were purchased from R&D Systems (Wiesbaden-Nordenstadt, Germany). The inhibitor BB-3644 was a kind gift from British Biotech Pharmaceuticals (Oxford, U.K.). ECL detection reagent was purchased from Anermsham Pharmacia Biotech (Freiburg, Germany). TLC plates were purchased from Merck (Darmstadt, Germany). The HD-de- rivative Lipoas was kindly donated by Dr. V. Diehl (University Hospital Cologne, Cologne, Germany). The large cellular anaplastic lymphoma cell line Karpas 299 was kindly provided by Dr. A. Karpas (Department of Pathology, Cambridge University, Cambridge, U.K.). The cells were cultured in RPMI 1640 supplemented with 10% FCS.

Alterations of the cellular cholesterol content

Cells undergoing cholesterol depletion or enrichment were washed and suspended in HBSS containing 1% BSA and then treated with MβCD for depletion or MβCD-cholesterol complex for enrichment at 37°C. The MβCD-cholesterol complex was prepared as previously described (18). Briefly, a solution of MβCD (40 mg/ml) in HBSS was added to cholesterol (2.5 mg/ml MβCD solution) in an Eppendorf tube and overlaid with N2 followed by overnight incubation at 30°C under gentle shaking. The solution was sterile filtered (0.2 μm) and stored at −20°C.

Lipid analysis

Cellular lipid composition was determined using methods previously described (26). Lipids were analyzed using charring densitometry. Briefly, samples were dissolved in 20 μl of chloroform. Defined dilutions of cholesterol were used as standards. The samples were applied to TLC plates that were developed in benzene/diethylether/acetatic acid (50:40:1, v/v/v). The plates were dried for 10 min at 180°C, allowed to cool, and then exposed to a solution of 10% copper sulfate in 8% aqueous phosphoric acid. The plates were subsequently rinsed, dried for 2 min at 110°C, and charred at 175°C for 10 min until lipid bands became visible. After scanning the TLC plates, lipids were identified by using an internal standard (cholesterol; Rf = 0.36) (dicyaglycerol (DAG); Rf = 0.51). Lipids were quantified by measuring the ODs using the PCBAS program. Results are either given as OD ratios of cholesterol/DAG, or given as micrograms per 106 cells after comparison with an internal cholesterol standard.

Determination of membrane-bound CD30 and CD70 by flow cytometry

FITC labeling was performed as follows: Abs (1 mg/ml; 0.5 ml) in 0.1 M NaHCO3/0.1 M NaCl (pH 9.2) were incubated with 1.5 mg of FITC/cellite for 30 min. The pellet-free supernatant was applied to a 2-25 desalting column to remove unconjugated material. Then, cells were stained with saturating amounts of anti-CD30 or anti-CD70 mAb (Ki-3-FITC or Ki-24-FITC, respectively) by 30-min incubation on ice in PBS containing BSA (0.1%) and propidium iodide (25 ng/ml) for the recognition and gating of dead cells. Cells were analyzed using a FACS Calibur flow cytometer (BD Biosciences, Heidelberg, Germany).

Peroxidase labeling of Abs

For labeling of Abs with HRP, 0.2 ml of an aqueous sodium periodate solution (21.4 mg/ml) was added to 1 ml of a solution of penstaden (1 mg/ml in distilled water) and incubated for 20 min at 25°C. The solution was dialyzed overnight against sodium acetate buffer (1 mM; pH 4.4) at 4°C. One milliliter of Ab solution (2 mg/ml) was added, and the reaction was started after addition of 40 μl of sodium carbonate buffer (0.5 M; pH 9.5). After 2 h of incubation at 25°C, 0.1 ml of sodium borohydride solution (2 mg/ml) in distilled water was added and incubated for another 2 h at 4°C. For stabilization, BSA (final concentration, 0.1 mg/ml) and a few grains of thymol were added. Then, the Abs were stored at −80°C.

Determination of sCD30

Flexible 96-well microtiter plates (Maxisorb, polar surface; Nunc; Life Technologies, Karlsruhe, Germany) were coated with 50 μl of Ki-2 mAb (50 μg/ml in sodium carbonate buffer (50 mM; pH 9.2)) by overnight incubation at 4°C. The plates were washed, blocked with PBS containing 5% milk powder for 1 h at room temperature, and subsequently stored at −20°C until use. After triple wash with PBS containing 0.1% Tween 20, serial dilutions of both the sCD30 standard (1320 U/ml) and the sCD30-containing samples were added and incubated for 2 h at room temperature. Plates were washed three times, and 50 μl of peroxidase-coupled Ki-3 mAb was added and incubated for 1 h at room temperature. After washing three times with PBS containing 0.1% Tween 20 and one time in PBS, 50 μl of ABTS solution (Roche Diagnostics) was added. After 1 h of incubation, the plates were evaluated at 405 nm in an ELISA reader. Samples were compared with the internal sCD30 standard.

Immunoprecipitation of sCD30

For immunoprecipitation of sCD30 from culture supernatants, protein A-agarose beads were coupled to 5 μl of Ki-1 mAb in 30 mM Tris-HCl (pH 8.5), 120 mM NaCl, and 0.1% Triton X-100 for 20 min at 4°C. After washing (three times) in the buffer, beads were incubated for 20 min at 4°C with the supernatants and washed again. The beads were centrifuged, and the supernatant was discarded. Beads were then heated with nonreducing SDS-PAGE loading buffer for 5 min at 95°C and subsequently run on SDS-PAGE (4–15% acrylamide). Gels were blotted on nitrocellulose membrane and stained with a mixture of peroxidase-labeled mAbs Ki-2 and Ki-4. Immunoreactive bands were detected by chemiluminescence with ECL detection reagent.

Isolation and analysis of lipid rafts

Membrane rafts were prepared as previously described with minor modifications (27). Briefly, 3 × 107 cells were washed in PBS and solubilized in 0.5 ml of TNE buffer (25 mM Tris-HCl (pH 7.5), 150 mM NaCl, 5 mM EDTA, complete protease inhibitor, and 1 mM sodium orthovanadate) containing 1% Triton X-100 and incubated for 30 min on ice. Cells were thoroughly suspended with a yellow 200-μl tip, and an equal volume of 80% sucrose (w/v in TNE) was mixed with the lysate. It was overlaid with 6 ml of 35% sucrose (w/v in TNE) followed by 3.5 ml of 5% sucrose (w/v in TNE). The gradient was centrifuged with a SW41 rotor at 4°C for 20 h at 40,000 rpm. Eleven gradient fractions (1 ml each) were harvested from the top. Fractions 4 and 10 were discarded, and 100 μl of fractions 5–11 were heated for 5 min at 95°C with 100 μl of double-concentrated nonreducing SDS-PAGE buffer (0.25 mM Tris-HCl (pH 6.8), 40% glycerol, 0.02% bromphenol blue, and 2% SDS). After protein separation by SDS-PAGE, gels were blotted on nitrocellulose membranes and stained with HRP-labeled anti-TACE Ab (MAB9302) or a mixture of HRP-labeled anti-CD30 Abs (Ki-2 mAb and Ki-4 mAb). Immunoreactive bands were detected by chemiluminescence with ECL detection reagent. Two microliters of each fraction were dot blotted on a polyvinylidene difluoride membrane and stained with peroxidase-coupled cholera toxin subunit B as a control of successful raft isolation.

Results

Cholesterol depletion with MβCD induces shedding of CD30 in lymphoma cells

To study the influence of the cellular cholesterol level on CD30 shedding, we treated the CD30+ ALCL-derived cell line Karpas 299 with MβCD, which is known to decrease the cellular cholesterol level and to disrupt lipid rafts. The influence of MβCD on the surface expression of CD30 was measured by flow cytometry. As shown in Fig. 1A, surface expression of CD30 decreased upon a 30-min MβCD treatment (3 mM). The decrease continued when MβCD was removed after 30 min, and cells were further incubated (Fig. 1B). To investigate whether this loss of membrane-bound
CD30 was due to CD30 shedding, we analyzed sCD30 in the supernatant of MβCD-treated cells. In accordance with the flow cytometry, there was a dose-dependent MβCD-induced increase of sCD30 in the supernatants (Fig. 1C), corroborating that this effect was caused by shedding.

Next, we studied the effect of MβCD on the Hodgkin-derived cell line L428. The cell surface expression of CD30 decreased in a time- (data not shown) and dose-dependent way (Fig. 1D). As a control, we measured the surface expression of CD70, which was not altered by MβCD treatment (Fig. 1E). To investigate the integrity of the shedding product, sCD30 from the culture supernatant was immunoprecipitated and evaluated by Western blotting (Fig. 1F). In this study, the sCD30 generated in response to MβCD treatment had the same molecular mass as constitutively generated 90-kDa sCD30.

To exclude nonspecific effects of MβCD, we measured cellular lipid levels of L428 cells after treatment with MβCD. As shown in Fig. 2, A and B, cellular cholesterol could be removed by treatment with MβCD in a dose-dependent way. As a control, we measured cellular DAG, which was not affected. The loss of cellular cholesterol correlated with an increase of sCD30 in the culture supernatant (Fig. 2C). MβCD treatment of Karpas 299 cells had similar effects on cellular lipids (Fig. 2D).

As a control, we treated Karpas 299 and L428 cells with MβCD, saturated with cholesterol. In this case, we did not observe any effect on CD30 shedding as shown by sCD30 ELISA (Fig. 2E). To find out whether the cholesterol-dependent CD30 shedding was restricted to lymphoma cells, HEK 293 cells were transfected with CD30 cDNA. However, in this respect, HEK 293 cells behaved like lymphoma cells, because MβCD, but not the MβCD-cholesterol complex, was able to induce CD30 shedding (data not shown).

Inhibition profile of the cholesterol-dependent CD30 sheddase

TACE has been identified as the main sheddase for phorbol ester-stimulated shedding of CD30 (17). To identify the responsible protease for cholesterol-dependent CD30 shedding, we tried to determine the inhibition profile of MβCD-induced shedding in L428 cells. TIMPs were used to pursue this objective. As shown by sCD30 ELISA, the shedding was efficiently blocked by TIMP-3.
(Fig. 3A), whereas TIMP-1 showed no effect on the MβCD-dependent release of sCD30 (B). Monitoring the cell surface expression of CD30 by flow cytometry revealed the same results (data not shown). Because this is the inhibition profile of TACE (28), it is very likely that TACE is the main enzyme of cholesterol-dependent CD30 shedding.

**Filipin, lovastatin, or cholesterol oxidase induces CD30 shedding**

We also examined the effect of filipin on CD30 shedding in L428 cells. Filipin is a sterol-binding polyene antibiotic (18) that disrupts lipid rafts. As shown by flow cytometry, a down-regulation of membrane-anchored CD30 (Fig. 4A) could be measured after filipin treatment. As a control, surface expression of CD70 remained unchanged (Fig. 4B). Because the broad spectrum metalloproteinase inhibitor BB-3644 showed inhibition on the filipin-dependent generation of sCD30 as shown in sCD30-ELISA (Fig. 4C), we concluded that metalloproteinase-dependent shedding was responsible for this effect.

For the inhibition of cellular cholesterol synthesis, cells were cultured with lovastatin, a potent inhibitor of 3-hydroxy-3-methylglutaryl-CoA reductase. Forty-eight-hour incubation with 15
μg/ml lovastatin led to a strong down-regulation of surface CD30 as determined by flow cytometry, and BB-3644 inhibited this loss (Fig. 5, A and B). We measured triplicate values of cellular lipids as shown in Fig. 5C, which revealed a significant decrease of cholesterol as shown by an unpaired t test (p = 0.035).

Oxidation of cellular cholesterol is catalyzed by cholesterol oxidase, which also leads to a degradation of lipid rafts (29). Incubation of L428 cells with cholesterol oxidase (4 U/ml for 20 h) caused a metalloproteinase-dependent down-regulation of CD30 (Fig. 5D) as demonstrated by flow cytometry and coincubation with BB-3644. A lipid analysis revealed a decrease of cellular cholesterol (Fig. 5E).

Localization of CD30 and TACE in detergent-soluble and -insoluble membrane compartments

It has been shown that the localization of APP inside or outside of lipid rafts plays a crucial role for its shedding. To investigate the respective localization of CD30 and TACE, we analyzed their solubility in Triton X-100 at 4°C, because lipid raft proteins are defined as being detergent insoluble at 4°C. We detected two bands corresponding to the precursor and the mature form of CD30 in the CD30 immunoblots (30) as well as two bands of TACE in the TACE immunoblot, the latter most likely representing the inactive pro-TACE and the smaller activated form of TACE, which lacks the prodomain (31). After lysis of L428 cells in 1% Triton X-100 and separation in a sucrose gradient, CD30 was partially detected in detergent-insoluble low-density complexes (Fig. 6A), but TACE was totally excluded from lipid rafts (B). As a positive control of successful raft isolation, we used peroxidase-linked cholera toxin subunit B, which binds to the raft-resident sphingolipid GM1 (Fig. 6C).

Depletion of cellular cholesterol by MβCD prevents the association of most proteins with lipid rafts (32). To show a modified distribution of CD30 after raft depletion, we treated L428 cells with MβCD and separated the detergent-insoluble fractions in a sucrose gradient. In untreated L428 cells, CD30 was partially localized in the raft fractions, but after MβCD treatment, CD30 was almost completely localized in high-density fractions (Fig. 6D), suggesting an increased probability of enzyme-to-substrate contact as a mechanism for cholesterol-dependent shedding of CD30. As a control, we examined whether the shedding-stimulating agent PMA had an effect on the distribution of CD30 in rafts and incubated cells with PMA for 5 and 60 min. As shown in Fig. 6D, we did not observe any altered distribution.

FIGURE 3. Inhibition profile of the cholesterol-dependent CD30 sheddase. L428 cells (10^6 per milliliter) were incubated with MβCD and/or different inhibitors as indicated for 60 min. A, After incubation with MβCD (8 mM) and/or different concentrations of TIMP-3, sCD30 was determined in the cell supernatants. The results show units per milliliter as means ± SD for triplicate determinations. B, After incubation with MβCD (8 mM) and/or TIMP-1 (485 nM ≈ 1 μg/ml), sCD30 was determined in the cell supernatants. The results show units per milliliter as means ± SD for triplicate determinations.

FIGURE 4. Stimulation of CD30 shedding by filipin in L428 cells. A–C, L428 cells (10^6 per milliliter) were incubated in the absence or presence of filipin (400 ng/ml) and/or BB-3644 (5 μM) for 60 min. Then, membrane-bound CD30 (A) or CD70 (as a control) (B) was determined by flow cytometry. C, sCD30 in the supernatants was measured. The results show units per milliliter as means ± SD for quadruplicate determinations.
Discussion

Our results showed that CD30 shedding is stimulated by different agents such as MβCD, filipin, lovastatin, and cholesterol oxidase. Shedding stimulation was time- and dose-dependent and correlated with the reduction of cellular cholesterol, suggesting that the increased CD30 shedding is indeed due to cholesterol depletion and not to another effect of the drugs. Because this effect could be induced in cell lines of different origin such as the Hodgkin-derived L428, the ALCL-derived Karpas 299, as well as the CD30-transfected HEK 293 cells, it appears to be a general phenomenon, which is not restricted to specific cell types.

As evidenced by the inhibitory effect of BB-3644, metalloproteinases were identified to cause the cholesterol-dependent shedding of CD30, and by evaluating the inhibition profile by the inhibitors TIMP-3 and TIMP-1, we suggest TACE as the leading CD30 sheddase (33, 34). So far, TACE is the only described metalloproteinase with this inhibition profile, but we cannot totally rule out the possibility of the involvement of another metalloproteinase. Thus, cholesterol-dependent shedding of CD30 probably involves the same proteolytic enzyme as previously described for the shedding of CD30 (17).

Cholesterol depletion was shown to stimulate APP cleavage by an effect on the α-secretase termed a disintegrin and metalloproteinase-10 (18). Recently, new light was shed on this process, and the existence of two APP pools was suggested. APP inside lipid rafts seemed to be cleaved by β-secretase, whereas APP outside rafts was shed by α-secretase (19). Both pathways could be stimulated: Raft recruitment of APP by cross-linking led to enhanced β-cleavage, whereas raft depletion by MβCD increased α-cleavage. Therefore, β-amyloid peptide generation may depend on interactions of APP with lipid rafts. By separation of cellular lipid rafts with a Triton X-100-based method, we demonstrated for the first time that CD30 partially distributes to the raft fractions, whereas TACE was excluded from rafts. How the distribution of CD30 to raft and nonraft fractions is regulated is not clear, but many surface receptors such as T and B cell receptors dimerize or...
noblotted with peroxidase-labeled anti-CD30 Ki-2/Ki-4 mAb. (nonraft fractions) were separated by nonreducing SDS-PAGE and immunoblotted under nonreducing conditions. The standard (75 kDa) for determination of TACE. for determination of CD30 and commercially available TACE ectodomain sucrose gradient and immunoblotted under nonreducing conditions. The ° 2H11003 containing 0.1% BSA in the absence or presence of M 2H9252 rafts of 3° 2H11003 – 3 – 5 (raft fractions) and 9 – 11 (nonraft fractions) were separated by nonreducing SDS-PAGE and immunoblotted with HRP-labeled anti-CD30 Ki-2 and Ki-4 mAb. B. Proteins of fractions 3–5 (raft fractions) and 9–11 (nonraft fractions) were separated by nonreducing SDS-PAGE and immunoblotted with HRP-labeled anti-TACE MAB9302 mAb. As a control, the TACE-ectodomain standard was used. C. Two microliters of each fraction of L428 cells were dot blotted and stained with peroxidase-labeled cholera toxin subunit B as a positive control for lipid rafts. In fractions 3–5, rafts were isolated. D. L428 cells (1.5 × 10^7 per milliliter) were incubated in prewarmed RPMI 1640 containing 0.1% BSA in the absence or presence of MβCD (10 mM) or PMA (5 ng/ml) at 37°C for 5 or 60 min as indicated. Membrane rafts of 3 × 10^7 cells were prepared, and proteins of fractions 3–5 (raft fractions) and 9–11 (nonraft fractions) were separated by nonreducing SDS-PAGE and immunoblotted with peroxidase-labeled anti-CD30 Ki-2/Ki-4 mAb.

FIGURE 6. Localization of CD30 in membrane rafts. A–C, Membrane rafts of 3 × 10^7 L428 cells were isolated by applying cell lysates to a sucrose gradient and immunoblotted under nonreducing conditions. The indicated molecular mass markers were internal sCD30 standard (90 kDa) for determination of CD30 and commercially available TACE ectodomain standard (75 kDa) for determination of TACE. A. Proteins of fractions 3–11 were separated by nonreducing SDS-PAGE and immunoblotted with a mixture of HRP-labeled anti-CD30 Ki-2 and Ki-4 mAb. B. Proteins of fractions 3–5 (raft fractions) and 9–11 (nonraft fractions) were separated by nonreducing SDS-PAGE and immunoblotted with HRP-labeled anti-TACE MAB9302 mAb. As a control, the TACE-ectodomain standard was used. C. Two microliters of each fraction of L428 cells were dot blotted and stained with peroxidase-labeled cholera toxin subunit B as a positive control for lipid rafts. In fractions 3–5, rafts were isolated. D. L428 cells (1.5 × 10^7 per milliliter) were incubated in prewarmed RPMI 1640 containing 0.1% BSA in the absence or presence of MβCD (10 mM) or PMA (5 ng/ml) at 37°C for 5 or 60 min as indicated. Membrane rafts of 3 × 10^7 cells were prepared, and proteins of fractions 3–5 (raft fractions) and 9–11 (nonraft fractions) were separated by nonreducing SDS-PAGE and immunoblotted with peroxidase-labeled anti-CD30 Ki-2/Ki-4 mAb.

oligomerize after ligand binding, which has been shown to increase association with rafts (35). For CD30, self-association and oligomerization have been demonstrated (36) and might be important for regulating the raft association of CD30. We showed that, after cholesterol depletion, CD30 was no longer present in the low-density lipid rafts. This might possibly increase the access-

bility of the CD30 cleavage site to TACE or another nonraft protease and thus provides a possible mechanism that participates in cholesterol-dependent CD30 shedding. Another possible explanation is provided by the strongly increased phase separation and the large areas of lipid-ordered and -disordered states that have been described in cholesterol-depleted cells (37). For cholesterol-dependent shedding of IL-6R, the formation of larger subdomains of similar lipid order status in cholesterol-depleted cells has been suggested as a mechanism that would lead to a facilitating association of substrate and enzyme (20). A conceivable mechanism may also be the existence of an accessory raft-dependent protein that is required to present CD30 to the sheddase, which would become available following raft depletion. It has been described that cholesterol may stabilize receptors in defined conformations related to their biological functions (38). Therefore, it is also possible that the sheddase is intrinsically more active in the absence of cholesterol.

Statins, known as inhibitors of 3-hydroxy-3-methylglutaryl-CoA reductase and therefore being cholesterol-lowering drugs, are used for the investigation of cholesterol-dependent shedding (18). In addition, they may be beneficial in the treatment of inflammatory diseases (39), especially on Th1-related diseases. Oral atorvastatin reversed paralysis in a mouse model for multiple sclerosis and promoted a Th2 bias (40, 41). Simvastatin possessed anti-inflammatory properties in a Th1-driven model of murine inflammatory arthritis (42). The pathogenesis of atherosclerosis, which is treated with statins, seems to be Th1 linked (43, 44). Recently, sCD30 has been shown to be important for the regulation of the immune system in vivo and in vitro (12, 45) and seems to play a counterregulatory role in the Th1-type immune response (11). Because serum levels of sCD30 were elevated in remittent phases of two Th1-driven diseases, such as multiple sclerosis and rheumatoid arthritis, it is tempting to speculate that the beneficial effect of cholesterol-lowering drugs on Th1-mediated diseases is connected to an increased generation of sCD30 in addition to other immunosuppressive effects of statins that have already been described (46). Whether or not cholesterol may become a direct target for modulation of the immune system remains to be elucidated in the future.

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