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Activated Human T Lymphocytes Express a Functional C3a Receptor

Thomas Werfel,2* Konstanze Kirchhoff,* Miriam Wittmann,* Gabriele Begemann,* Alexander Kapp,* Feodor Heidenreich,† Otto Götte,‡ and Jörg Zwirner‡

The C3a molecule is an anaphylatoxin of the C system with a wide spectrum of proinflammatory effects predominantly on cells of myeloid origin. In this study we investigated the expression of the high affinity receptor for C3a (C3aR) in human T lymphocytes using receptor-specific mAb. C3aR expression was detected in CD4+ and CD8+ blood- or skin-derived T cell clones (TCC) from birch pollen-sensitized patients with atopic dermatitis. No significant difference in C3aR expression in CD4+ or CD8+ TCCs could be observed. In contrast to C3a(desArg), C3a led to a transient calcium flux in TCCs expressing the C3aR, whereas C3aR-negative TCCs were unreactive. Circulating T cells from patients suffering from severe inflammatory skin diseases expressed the C3aR, whereas no expression of C3aR could be found in unstimulated T lymphocytes from patients with mild inflammatory skin diseases or from healthy individuals. Type I IFNs, which are potent stimulators of cellular immunity, were identified as up-regulators of C3aR expression in vitro in freshly isolated or cloned T lymphocytes. Moreover, C3aR+ T cells were found at the sites of injection in IFN-β-treated patients with multiple sclerosis. These data provide direct evidence for the expression of C3aR on activated human T lymphocytes; this may point to a biological function of C3a in T cell-dependent diseases. The Journal of Immunology, 2000, 165: 6599–6605.

The C3a molecule is one of the anaphylatoxins of the C system, a family of factors comprising C3a, C4a, and C5a. A wide spectrum of C3a effects has been measured in vivo and in vitro (1). These include the release of histamine and intracellular calcium ions as well as chemotaxis in human mast cells and basophils (2–7). C3a is also a potent stimulus for human eosinophils, which respond to C3a with a mobilization of calcium ions, superoxide anion production, degranulation, chemotaxis, and adhesion to postcapillary venules (8–11). Furthermore, C3a induces the production of reactive oxygen species as well as the release of IL-8 and lysosomal enzymes from neutrophils (12, 13). In human monocytes and macrophages C3a induces the release of PGE2 and calcium ions (14, 15). In addition, C3a is a potent suppressor of both Ag-specific and polyclonal Ab responses through the induction of nonspecific suppressor T cells (16). The aforementioned functional responses are specific for C3a. Whenever tested, its natural catabolite C3a(desArg) was inactive (2, 4, 5, 8, 10, 11, 13–16).

The recently cloned human C3aR (3) belongs to the large family of G protein-coupled receptors with seven transmembrane segments (17–19). It represents the only as yet characterized receptor for C3a and does not bind C3a(desArg) (20). Northern blot analyses demonstrated that the C3aR is widely expressed in different tissues, including lymphoid organs, which suggests that anaphylatoxin C3a may play a central role in inflammatory processes (18).

In this study we demonstrate that T cell clones (TCCs) from patients with atopic dermatitis express the functional C3aR. Severe inflammation was a prerequisite for C3aR expression in circulating human T lymphocytes from patients with inflammatory skin diseases. Type I IFNs, which are known to induce a Th1 type of immune response, could be identified to induce C3aR expression in T lymphocytes in vivo as well as in vitro. Thus, C3aR expression in activated T lymphocytes provides a link between the C system as part of the innate immune system and the adaptive immune response, which depends on T lymphocytes.

Materials and Methods
Patients and healthy control persons

Lymphocytes were isolated from healthy blood donors or from healthy staff members of the Department of Dermatology and Allergology of the Hannover Medical University. Patients suffering from inflammatory skin diseases were treated in the Department of Dermatology and Allergology, and patients suffering from multiple sclerosis were treated with IFN-β1b (Betaseron; Schering, Berlin, Germany) in the Department of Neurology of Hannover Medical University. All patients gave their written consent for skin biopsies. The study was approved by the local ethics committee.

Isolation of T lymphocytes

Mononuclear cells were separated from whole blood of healthy donors by density gradient centrifugation on Lymphoprep (density = 1.077 g/cm3) (Nycomed Pharma, Oslo, Norway).

For the isolation of lymphocytes and monocytes, a JE-6B centrifuge (Beckman, Munich, Germany) equipped with an Elutriator rotor was used. The flow rate of the elution medium was adjusted to 18 ml/min. The centrifugation speed was reduced step by step from 3200 to 1900 rpm, and the cells were collected in 200-ml fractions. Cells emerging from the centrifuge were determined by flow cytometry (Becton Dickinson, Heidelberg, Germany). Most of the monocytes were enriched in the fraction obtained at a rotor speed of 2000 rpm. Platelets emerged at 2700 rpm or less, small lymphocytes at 2400 rpm, and a monocyte-lymphocyte mixture at 2150 rpm. The elution medium was PBS without Ca2+ or Mg2+ containing 2%
Purity was >98%.

Preparation of single cells from skin biopsies

Punch biopsies were taken from lesional skin. Epidermis and dermis were separated by overnight incubation in dispase (2.4 U/ml; Roche Molecular Biochemicals, Mannheim, Germany) at 4°C as described (21). The epidermis was incubated with 0.25% trypsin (Sigma-Aldrich, Deisenhofen, Germany) for 20 min at 37°C and washed in modified Hank’s solution. Dermal tissue was incubated in Hank’s solution (5 h at 37°C) containing collage- nase, DNase, dispase (Roche Molecular Biochemicals), hyaluronidase (Sigma-Aldrich), and 10% FCS. Dermal cell suspensions were washed and filtered through nylon gauze. Epidermal and dermal cell suspensions were mixed for subsequent cell culture procedures. The percentage of T cells was assessed by flow cytometry with an Ab to CD3 (Becton Dickinson). T cells represented <15% of all cells in cutaneous cell suspensions.

Investigation of TCCs

The generation of birch pollen-specific TCCs has been described recently (22). Cloning was performed by limiting dilution in the presence of the recombinant birch pollen Ags betv1 and betv2 (Biomy, Linz, Austria) and IL-2 (Roche Molecular Biochemicals). Responder cells from patients and a mixture of betv1 and betv2 Ags were added to limiting-dilution wells together with 2 × 10⁵ autologous APCs (PBMC, irradiated with 55 Gy using ¹³⁷Cs). The cultures were kept in Iscove’s medium supplemented with 4% heat-inactivated AB serum, and IL-2 (10 U/ml). After 12–14 days, wells with >50 lymphoblasts were scored as positive. Because cultures were only expanded from suspensions diluted to contain 0.3 proliferating cells or less, there was a high probability of clonal cell growth. Ag specificity of TCCs was tested in a restimulation assay (22) in the presence of betv1 or betv2 Ags and 7.5 × 10⁶ irradiated autologous PBMC.

Stimulation of T lymphocytes or TCCs with cytokines

The expression of the C3αR was tested after stimulation of T cells for 24 or 48 h with the following reagents: IL-2 (10–50 U/ml; Roche Molecular Biochemicals), IL-4 (4–10–50 ng/ml; R&D Systems, Wiesbaden-Norden- stadt, Germany), IFN-γ (200 U/ml; Genzyme, Rüsselsheim, Germany), IL-6 (20 ng/ml; R&D Systems), IL-8 (80 ng/ml; TEBU, Frankfurt, Ger- many), IL-10 (10 ng/ml; R&D Systems), IL-12 (50 ng/ml; R&D Systems), IL-16 (10 ng/ml; Genzyme), IFN-α (200–400 U/ml; Roche Molecular Biochemicals), IFN-β (200–400 U/ml; Biosource, Ratingen, Germany), IFN-γ (200 U/ml; Genzyme), TGF-β (100 U/ml; Roche Molecular Biochemicals) or TNF-α (200 U/ml; R&D Systems), PHA (10 μg/ml; Life Technologies, Eggenstein, Germany), Con A (10 μg/ml; Sigma-Aldrich), or PMA (20 ng/ml; Sigma-Aldrich).

Flow cytometric analyses of membrane molecules

Cells were analyzed by double-color immunofluorescence staining using a FACScan flow cytometer (Becton Dickinson). For indirect labeling, cells (2 × 10⁵) were washed and resuspended in PBS containing 0.2% gelatin, 20 mM sodium azide, and 10 μg/100 μl heat-aggregated human IgG (Sig- ma-Aldrich). Subsequently, cells were incubated with anti-C3αR mAb for 1 h on ice. In a second step, cells were incubated with a FITC-conjugated goat anti-mouse Ig Ab (Dianova, Hamburg, Germany) for another hour on ice. Cells were further treated with 0.5 mg/ml mouse IgG (30 min, 4°C; Sigma-Aldrich) to completely saturate all binding sites of the secondary Ab. Lymphocytes were then incubated with PE-labeled anti-CD3 mAb (Immunotech, Hamburg, Germany), anti-CD8, or anti-CD4 mAb (45 min, 4°C; Dakopatts, Hamburg, Germany). Stained cells were washed three times and fixed in PBS containing 1% paraformaldehyde.

Preparation of recombinant anaphylatoxins

Recombinant C3a and rC3a(desArg) were generated as described. Both recombinant molecules contained an additional N-terminal tag of 11 aa (15, 20). The addition of the amino terminal aa to rC3a is without influence on the functional activity of rC3a, as recombinant and serum-derived C3a stimulated the release of N-acetyl-b-D-glucosaminidase from dibutyryl-cAMP-treated U937 cells equally well (15). LTP was released from isolated rC3a fractions by polymyxin B coupled to a solid phase. The protein fractions were incubated on a shaker for 24 h at 4°C in 0.1 M Tris-HCl, pH 7.4, 0.15 M NaCl, 0.1% taurine, 0.01% NaN₃, 0.001% sodium azide, and 10 μg/ml urease (Sigma-Aldrich) in the presence of octyl-b-D-glucopyranoside (Calbiochem-Novabiochem, Bad Soden, Germany). After centrifugation, the whole procedure was repeated and the supernatants were dialyzed against pyrogen-free 0.9% NaCl. LPS concentrations were determined by the Limulus assay (Coastost Endotoxin; Pharmacia, Freiburg, Germany) according to the manufacturer’s protocol. The LPS concentration in functional assays performed with the rC3a prepara- tion treated in this way was 3.2 pg/μg C3a. Labeling of the rC3a protein with carboxyfluorescein-N-hydroxysuccinimidester (Roche Molecular Biochemicals) was performed as described (15).

mAbs against the human C3αR

The generation of mAbs with specificity for the second, large extracellular loop of the human C3αR has been described recently (23). Binding of mAb hC3αRZ1 to the human C3αR can be specifically blocked by an excess of the peptide NNHHDILKFD encompassing the epitope recognized by this Ab. The peptide does not affect the binding of mAb hC3αRZ3 to the human C3αR, as mAb hC3αRZ3 recognizes a second, independent epitope on the second, extracellular loop of the receptor.

Measurement of intracellular calcium fluxes [Ca²⁺], by flow cytometry

The loading procedure with Fluo-3-AM (Molecular Probes, Eugene, OR) was conducted in a modified way as described previously (21). Blood T lymphocytes were suspended at 1 × 10⁶/ml in PBS supplemented with Ca²⁺, Mg²⁺, and 0.1% BSA containing 10 μM Fluo-3-AM, prediluted in 1% DMSO (v/v) containing 37.5 μl Pluronic F-127 (Sigma-Aldrich), for 20 min at 37°C. To remove extracellular Fluo-3-AM, cells were washed twice. Finally, the cells were adjusted to 2.5 × 10⁶/ml in PBS containing 145 mM NaCl, 5 mM KCl, 1 mM CaCl₂, and 1 mM MgCl₂, and kept in the dark until used. Assessment of [Ca²⁺], was performed at 37°C using flow cytometry. The argon laser was set at 488 nm (excitation), and emitted light was measured at 530 nm using the logarithmic mode. After analysis of the basal fluorescence of the sample, the stimulus was added to the test tube through a 24-gauge needle during the aspiration of the cells into the flow cytometer and the fluorescence profiles were acquired. Therefore, single cell [Ca²⁺], could be monitored continuously.

mRNA isolation and reverse transcription

mRNA was isolated from 10⁶ T lymphocytes using an mRNA isolation kit (Roche Molecular Biochemicals) according to the supplier’s instructions. For RT-PCR analysis, RNA was subjected to first strand cDNA synthesis using Oligo(dT)₁₅ for full length cDNA synthesis. The RT reaction mixture contained final concentrations of 50 U Expand-RT (Roche Molecular Biochemicals) according to the supplier’s instructions. For RT-PCR analysis, RNA was subjected to first strand cDNA synthesis using Oligo(dT)₁₅ for full length cDNA synthesis. The RT reaction mixture contained final concentrations of 50 U Expand-RT (Roche Molecular Biochemicals), 10 mM DTT, 1 × first-strand RT buffer for Expand-RT, 0.5 mM of each dNTP (Roche Molecular Biochemicals), RNase inhibitor (Life Technologies, and 80 pmol Oligo(dT)₁₅, Roche Molecular Biochemicals). To control for genomic DNA contamination, cDNA synthesis was performed in the absence of reverse transcriptase. First strand cDNA was stored at −20°C.

PCR

For PCR amplification the resulting cDNA was amplified. PCR was performed as described previously (24). The following primers were used: CD3d sense 5’-CTG GAG CCT GGA AAA CGC ATG and antisense 5’-GTA GTG AGC ATC ATC TGC AGT, resulting in a 309-bp product; C3αR sense 5’-TGA AGC CTT CAG CTA TCT CAG and antisense 5’-GGA CAA TGA TGGA GGG GAT GAG, based on the published se- quence of the human C3αR (18). An aliquot of each PCR product was subjected to electrophoresis on a 2% agarose gel (Qualigold: AGS, Heidelberg, Germany), stained with ethidium bromide, visualized, and photographed under ultraviolet illumination.

Real-time fluorescence PCR

Real-time fluorescence PCR was performed using the LightCycler (Roche Molecular Biochemicals). For quantitative PCR the dsDNA binding dye SYBR Green (Roche Molecular Biochemicals) was used according to the supplier’s instructions. PCR was performed by rapid cycling in a reaction volume of 20 μl with 0.5 μM of each primer and 4 μl cDNA. As reaction buffer, the LightCycler DNA Master SYBR Green I (contain- ing reaction buffer, Taq DNA polymerase, dNTPs (with dUTP instead of dTTP), MgCl₂, and a calibrated amount of SYBR Green I dye (Roche Molecular Biochemicals) and additional MgCl₂ (the final concentration was 3.75 mM for CD3d and 3 mM for C3αR) was used. After an initial denaturation step at 95°C for 30 s, a amplification was performed using 35 cycles (CD3d) and 40 cycles (C3αR), respectively, of denaturation (95°C), annealing (60°C), and extension (72°C). Fluorescence was measured at the end of the annealing period of each cycle to monitor amplification. Real-time monitoring of the amplification allows quantitation of the samples.
during the log-linear phase of the PCR. As an internal standard was not coamplified, C3aR expression between the samples could only be compared in a semiquantitative manner.

After amplification was complete, a final melting curve was recorded by cooling the samples to 65°C at 20°C/s and then increasing the temperature to 95°C at 0.2°C/s. Fluorescence was measured continuously during the slow temperature rise to monitor the dissociation of the PCR product. The fluorescence signal was plotted in real time against the temperature to produce melting curves of each sample. Melting curves were then converted to melting peaks by plotting the negative derivative of the fluorescence with respect to temperature against temperature (dF/dT vs T). Thus, each specific PCR product generates a specific signal and, therefore, a product-specific melting peak.

**Immunohistology**

Immunohistology was performed as described elsewhere in detail (21). Briefly, tissue specimens obtained by punch biopsy were shock frozen in liquid nitrogen. Cryostat tissue sections (5-µm thick) were dried and then fixed for 10 min in acetone. The endogenous peroxidase of the cells was inhibited by an incubation for 15 min with 150 µl PBS (to which 3 ml 1 M sodium azide was added) and 0.5 ml peroxide (30%). After three washes in PBS, the fixed sections were incubated in 50% normal goat serum (Life Technologies, Karlsruhe, Germany) in PBS to block Fc receptors. The fixed sections were overlaid with a predetermined optimal concentration of anti-C3aR (hC3aRZ1) or anti-CD3 (Dakopatts) mAbs containing 2% goat serum or with corresponding concentrations of isotype control mAbs in the same buffer. After a 1-h incubation in a moist chamber and three washing steps in PBS the sections were overlaid with biotin-conjugated sheep antimouse Ig (Amersham, Braunschweig, Germany) diluted 1:400 for 40 min at room temperature followed by three washing steps in PBS. The sections were incubated for 30 min at room temperature with a 1:1000 dilution of streptavidin-peroxidase (Dianova), washed, and then stained by immersion in 150 ml chromogenic solution of 3-amino-9 ethyl carbazole (Sigma-Aldrich) containing N,N-dimethylformamide (Merck, Darmstadt, Germany) and 0.1 ml hydrogen peroxide (30%) for 8 min. The sections were counterstained with hemalum and then mounted in Faramount Mounting Medium (Dakopatts).

**Statistical analysis**

Statistical analyses were performed using the Wilcoxon signed rank test or the paired Student t test, as indicated.

**Results**

**Expression of the C3aR in human TCCs**

A binding of anti-C3αR mAb hC3aRZ1 was detected on CD8⁺ T cells that had been obtained from birch pollen-sensitized patients with atopic dermatitis (22) (Table I). The binding of anti-C3αR mAb hC3aRZ1 to TCCs was specific because it could be blocked with a 50-fold excess of the peptide NNHUDISLKFJD, which represents the C3αR epitope recognized by this mAb (Fig. 1). As expected, binding of mAb hC3aRZ3 was not inhibited by peptide NNHUDISLKFJD as this mAb recognizes a different epitope. The specificity of binding was also shown by down-modulation of the binding sites for anti-C3αR mAb hC3aRZ1 through preincubation of lymphocytes with C3α (Fig. 2). The expression of C3αR on TCCs was relatively stable: 26 of 36 C3αR⁺ TCCs that were tested for a second time after 8 wk were still C3αR⁺. Only 1 of 13 TCCs that were initially C3αR-negative bound low amounts of anti-C3αR mAb after 8 wk of culture. The extent of binding of FITC-conjugated C3α to TCCs correlated with the strength of anti-C3αR mAb binding (data not shown). There was no significant difference in the intensities of anti-C3αR mAb binding to CD4⁺ and CD8⁺ TCCs (data not shown).

C3α, in contrast to C3α(desArg), led to a transient calcium influx in C3αR⁺ TCCs (Fig. 3). No calcium influx was detectable after C3α incubation in C3αR-negative TCCs (data not shown). C3αR mRNA was detected in C3αR⁺ TCCs but not in feeder cells (Fig. 4). The feeder cells had been cultured in parallel to TCCs to exclude amplification of C3αR mRNA from irradiated monocytes.

**Expression of C3αR on freshly isolated T cells**

We found no expression of C3αR mRNA and no binding of anti-C3αR mAbs using freshly isolated, unstimulated T lymphocytes from healthy donors (data not shown). Circulating T cells from patients suffering from less severe skin diseases (n = 10), i.e., atopic dermatitis with a severity score of atopic dermatitis <30, mild chronic plaque psoriasis, bullous pemphigoid with few blisters, were C3αR-negative. One representative result from a patient with mild chronic plaque psoriasis is shown in Fig. 5A. In contrast, T cells from patients suffering from extensive and severe inflammatory skin diseases (n = 12) such as severe psoriasis erythroderma, pemphigus foliaceous, atopic dermatitis with severity score of atopic dermatitis >50, or erysipelas expressed C3αR mRNA as

### Table I. C3αR expression in TCCs from birch pollen-sensitized patients with atopic dermatitis

| C3αR Expression | CD4⁺ TCC | CD8⁺ TCC | TCC from Blood | TCC from Skin |
|-----------------|---------|---------|----------------|--------------|
| dMCF 0–1        | 8⁺      | 5       | 4              | 9            |
| dMCF 1–3.5      | 14      | 7       | 7              | 14           |
| dMCF 3.5–7.1    | 13      | 12      | 4              | 5            |

* Binding of anti-C3αR mAb hC3aRZ1 to CD4⁺ or CD8⁺ TCC derived from blood or skin was analyzed by flow cytometry. Binding intensity (dMCF) to CD3⁺ T cells was determined by MCF of the specific Ab minus MCF of the isotype control mAb.

* Number of TCCs.
detected by PCR (data not shown) and bound low amounts of anti-C3aR mAb ahC3aRZ1. One representative result from a patient with psoriasis erythroderma is shown in Fig. 5B. This binding proved to be specific because it could be blocked by peptide NNHUDISLKFJD or by preincubation of the T cells with C3a (data not shown). Monoclonal Ab hC3aRZ3, which recognizes a second epitope of the C3aR (23), was tested in parallel on 22 PBMC fractions from patients with mild or severe inflammatory skin diseases. The binding intensities of mAbs hC3aRZ1 and hC3aRZ3 as determined by the median channel fluorescence (dMCF) of the specific Ab minus MCF of the isotype control mAb correlated well (r = 0.63, p < 0.002; Spearman Rank correlation).

C3aR is induced by type I IFNs
C3aR mRNA was detected in isolated T cells that had been cultured for at least 5 h. A low binding of anti-C3aR mAbs was found on cultured, isolated T cells and on T cells cultured in PBMC preparations for at least 24 h. This binding increased during the following 2 days (data not shown). Type I IFNs were identified as stimulators of C3aR expression on freshly isolated T cells (Table II). Other tested cytokines (IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-16, TGF-β, TNF-α, and TNF-β) had no effect on C3aR expression in T cells (data not shown). The incubation of TCCs for 48 h with type I IFNs led to an increase in C3aR expression (Table III). Other cytokines (IL-2, IL-4, IL-5, IFN-γ, IL-10, IL-12, and IL-16) or PHA (10 μg/ml), Con A (10 μg/ml), or PMA (20 ng/ml) plus Ca-ionophore (250 ng/ml) were without effect on C3aR expression in TCCs (data not shown). In addition, we could demonstrate an increase in C3aR mRNA expression in TCCs by quantitative real time PCR (Fig. 6) after incubation with IFN-α for 5 h.

Interestingly, we found a significantly higher IFN-α concentration in the supernatants of TCCs with a high expression of C3aR (i.e., dMCF > 3.5) as compared with TCCs with no or low C3aR expression (median IFN-α = 0.7 pg/ml (0.5–1.0 pg/ml, n = 7) vs 2.2 pg/ml (1.0–3.2 pg/ml, n = 9); p < 0.01; Wilcoxon Rank test).

An induction of C3aR expression by IFN-β could be demonstrated in vivo. Treatment of patients suffering from multiple sclerosis with IFN-β led to a detectable expression of C3aR protein in lymphocytes infiltrating the skin at the sites of IFN-β injection (Fig. 7).
could be confirmed in vitro.

The regulatory effect of type I IFNs on C3aR expression in lymphocytes local inflammatory conditions expressed the C3aR protein. A stimulative effect of type I IFNs on C3aR expression in human T lymphocytes. A detailed analysis of a high number of TCCs showed that the receptor can be expressed on CD4⁺ and CD8⁺ TCCs obtained from both skin and blood. The specificity of our findings was shown by blocking the binding of the C3aR-specific mAb hC3aRZ1 to T cells using a peptide representing the C3aR epitope recognized by the Ab. In addition, reduction of Ab binding could be demonstrated following preincubation of the T cells with the C3a ligand. Down-modulation of receptor expression upon exposure to the ligand is a well-documented phenomenon for members of the family of G protein-coupled receptors with seven transmembrane segments and has also been demonstrated for the C3aR (25). The functional coupling of the C3aR to intracellular activation pathways in T lymphocytes was shown by C3a-induced calcium fluxes, which had previously been demonstrated in C3aR⁺ monocytes/macrophages and granulocytes (5, 15, 26).

The results of this study further demonstrate that expression of the receptor for the anaphylatoxin C3a can be induced under inflammatory conditions in vitro and in vivo. T lymphocytes from patients with signs of severe systemic inflammatory reactions clearly expressed C3aR. The investigation of lymphocytes obtained from sites of IFN-β injection in patients suffering from multiple sclerosis revealed that T cells accumulating under these local inflammatory conditions expressed the C3aR protein. A stimulative effect of type I IFNs on C3aR expression in lymphocytes could be confirmed in vitro.

Discussion

This is the first report that provides experimental evidence for the expression of the C3aR in human T lymphocytes. A detailed analysis of a high number of TCCs showed that the receptor can be expressed on CD4⁺ and CD8⁺ TCCs obtained from both skin and blood. The specificity of our findings was shown by blocking the binding of the C3aR-specific mAb hC3aRZ1 to T cells using a peptide representing the C3aR epitope recognized by the Ab. In addition, reduction of Ab binding could be demonstrated following preincubation of the T cells with the C3a ligand. Down-modulation of receptor expression upon exposure to the ligand is a well-documented phenomenon for members of the family of G protein-coupled receptors with seven transmembrane segments and has also been demonstrated for the C3aR (25). The functional coupling of the C3aR to intracellular activation pathways in T lymphocytes was shown by C3a-induced calcium fluxes, which had previously been demonstrated in C3aR⁺ monocytes/macrophages and granulocytes (5, 15, 26).

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Thus, C3a may link lymphocyte-driven immune responses to the innate immune system, an important part of which is C. This link is emphasized by the ability of T lymphocytes to synthesize and secrete properdin as well as C3 (27, 28). These molecules together with factor B build the alternative C3 convertase that generates C3a. The local accumulation of C3a may directly affect T cell functions following the induction of C3aR on these cells. Type I IFNs play an important role in shifting T cell differentiation toward a Th1 type of immune response (29). The role of C3a in type I immune responses has yet to be elucidated. C3 preparations containing C3a inhibit human lymphocyte blastogenesis and the generation of CTL (30, 31). It may be speculated that type I immune responses are mediated by the inhibitory effects of C3a on activated T lymphocytes.

The anaphylatoxin C3a has also been implicated in the suppression of human and murine in vitro Ab responses, whereas C3a(desArg) was inactive (16, 32). Subsequently, it was suggested that the C3a-induced release of PGE₂ from macrophages could be a major element in the immunosuppression induced by C3a (14). Our results point to the possibility that a direct interaction of activated T cells with C3a may be involved in C3a-mediated immunosuppression.

Immunosuppressive effects by C3a on the polyclonal Ab response and on the cytokine synthesis in human B lymphocytes have also been reported previously (33). Signaling through the

Table II. Induction of C3aR epitopes by IFN-α on lymphocytes isolated from peripheral blood

|                  | mAb hC3aRZ1 | mAb hC3aRZ3 |
|------------------|-------------|-------------|
| Medium           | 2.0 ± 0.4   | 4.5 ± 0.4   |
| IFN-α            | 3.3 ± 0.7⁶  | 6.9 ± 0.6⁶  |

* Freshly isolated T lymphocytes (n = 7) were incubated with IFN-α for 48 h and analyzed by flow cytometry. Binding intensities (dMCF ± SEM) of mAbs hC3aRZ1 and hC3aRZ3 to CD3⁺ T cells were determined by MCF of the specific Ab minus MCF of the isotype control mAb.

† p < 0.005; Wilcoxon signed rank test.

‡ p < 0.05; paired t test.

Table III. Induction of C3aR expression in TCC by type I IFNs

|                  | mAb hC3aRZ1 | mAb hC3aRZ3 |
|------------------|-------------|-------------|
| Stimulation of TCC with 200 U/ml IFN-α or 200 U/ml IFN-β (n = 7) |
| Medium           | 5.8 ± 1.6   | 4.2 ± 1.0   |
| IFN-α            | 8.3 ± 2.0⁶  | 5.4 ± 1.3⁶  |
| IFN-β            | 9.0 ± 2.2⁶  | 5.5 ± 1.4⁶  |
| Stimulation of TCC with 1500 U/ml IFN-α (n = 12) |
| Medium           | 3.5 ± 0.7   | 2.1 ± 0.3   |
| IFN-α            | 6.2 ± 1.1⁶  | 3.8 ± 0.6⁶  |

* T lymphocytes were incubated with different concentrations of IFN-α or -β for 48 h and analyzed by flow cytometry. The binding intensities (dMCF ± SEM) of mAbs hC3aRZ1 and hC3aRZ3 to CD3⁺ T cells were determined by MCF of the specific Ab minus MCF of the isotype control mAb.

† p < 0.01; Wilcoxon signed rank test.

‡ p < 0.005; paired t test.

FIGURE 6. IFN-α induces expression of C3aR mRNA in human T lymphocytes. Real-time PCR was performed for the detection of C3aR and CD3β mRNA expression in highly purified T lymphocytes after a 5-h culture in the presence or absence of IFN-α. Fluorescence intensity of the dsDNA binding dye SYBR Green was plotted vs cycle numbers. Fluorescence signals were acquired at the end of the annealing period of each cycle.
C3αR expressed on B lymphocytes was suggested to account for these results although C3α(des Arg) was as effective as C3α. However, C3α(des Arg) does not bind to or signal through the C3αR (20). Therefore, T lymphocytes use the C3αR for the transmission of C3α-dependent signals, whereas in the case of B cells this has not been conclusively shown.

Interestingly, expression of the receptor for the anaphylatoxin C5α, which was demonstrated on a subpopulation of resting T lymphocytes, was up-regulated after PHA stimulation (34). C5α, in contrast to C3α, may be regarded as a stimulator of the immune response. It has been shown to elicit a broad range of effects in cells of the myeloid lineage (35). C5α also enhances humoral and T cell-mediated immune responses, with macrophages being important effector cells (36, 37). Although the C3αR was readily detectable on activated T lymphocytes, no expression of C5αR protein was found in our experimental setting using different C5αR-specific mAbs (data not shown). Thus, on the level of the T lymphocyte, C3α may represent the dominant regulatory anaphylatoxin.

Our results provide direct evidence for the expression of a functional C3αR in activated T lymphocytes in vitro and in vivo, which points to a biological function of C3α in T cell-regulated diseases.

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