International Immunology, Vol. 12, No. 4, pp. 547–554

Stimulation of FcγR receptors induces monocyte chemoattractant protein-1 in the human monocytic cell line THP-1 by a mechanism involving IκB-α degradation and formation of p50/p65 NF-κB/Rel complexes

Andrés Alonso, Yolanda Bayón, Marta Renedo and **Mariano Sánchez Crespo**

Instituto de Biología y Genética Molecular, Consejo Superior de Investigaciones Científicas, Facultad de Medicina, 47005 Valladolid, Spain

Keywords: chemokines, Fc receptors, inflammation, monocytes/macrophages, transcription factors

Abstract

THP-1 monocytic/macrophage cells were stimulated via their FcγR receptors with insoluble aggregates of human IgG and the production of the C–C chemokine monocyte chemoattractant protein (MCP)-1 assayed. A dose- and time-dependent production of MCP-1 comparable to that produced by the most potent agonists could be detected in the culture medium by a sensitive ELISA assay. This was accompanied by a parallel activation of the transcription factor NF-κB as judged from both the appearance of κB-binding activity containing p50/p65 NF-κB/Rel complexes in the nuclear extract and the disappearance of the NF-κB inhibitor IκB-α in the cell lysate. In contrast, IκB-β and IκB-ε expression was not modified, thus pointing to the occurrence of a selective degradation of IκB-α under those conditions. Attempts to modulate MCP-1 production with compounds that display inhibitory effects on the activation of NF-κB such as the proteasome inhibitor *N*-acetyl-leucinyl-leucinyl-norleucinal, the antioxidant pyrrolidine dithiocarbamate and the salicylate derivative 2-hydroxy-4-trifluoromethylbenzoic acid showed a parallel effect on both MCP-1 production and NF-κB activation, thus pointing to the involvement of κB-binding sites on the transcriptional regulation of MCP-1 production. Our findings suggest the existence in monocytic cells of a signaling mechanism initiated by cross-linking of low-affinity FcγR, most likely of the FcγRII family since THP-1 cells do not express FcγRIII receptors, that involves activation of NF-κB associated to the proteolytic degradation of IκB-α and leads to the transcriptional up-regulation of MCP-1.

Introduction

Accumulation of leukocytes in tissues is a central event in the production of tissue injury in immune complex (IC)-mediated diseases such as arthritis, glomerulonephritis and immune vasculitis, and is causally linked to the generation of molecules which specifically recruit blood leukocytes. Interestingly, recent reports have assigned a central role in tissue injury to effector mechanisms linked to the activation of the receptors for the Fc portion of the antibody molecule (FcγR), in light of recent studies in murine strains with targeted disruption of the γ chain of FcγR, where it has been possible to uncouple IC deposition from the subsequent inflammatory response, e.g. the reduced incidence of proteinuria in NZB/NZW mice with the spontaneous model of lupus nephritis (1) and the absence of leukocyte infiltration in the reverse passive Arthus model (2–4). A connection between FcγR cross-linking and the generation of leukocyte chemoattractants has previously been reported by showing the induction of chemokines of the C–X–C subfamily specifically attracting polymorphonuclear leukocytes in both rat macrophages (5) and human leukocytes (6). However, polymorphonuclear leukocytes display a short survival in tissues and their presence is associated to acute injury, whereas chronic inflammatory damage requires the

Correspondence to: M. Sánchez Crespo

Transmitting editor: E. R. Unanue *Received* 1 October 1999, *accepted* 7 January 2000

recruitment of monocytes, which are specifically attracted by monocyte chemoattractant protein (MCP)-1 (7), a member of the C–C chemokine subfamily, which has been linked to a number of pathological processes such as glomerulonephritis (8) and rheumatoid arthritis (9), and constitutes the most potent chemoattractant detected in macrophage-rich atherosclerotic plaques (10). MCP-1 is expressed by a variety of cell types, including monocytes, fibroblasts, vascular endothelial cells and smooth muscle cells in response to different stimuli including tumor necrosis factor (TNF)-α, IL-1β, IFN-γ and phorbol ester, thus suggesting a complex mechanism of transcriptional regulation in which *trans*-acting factors binding to κB, TRE 12-α-tetradecanoylphorbol 13-acetate-response element (TRE) (11,12), IFN-γ-activated site (GAS) (13) and Sp1 *cis*-acting elements are involved (12).

In spite of the clear association between FcγR stimulation and immune effector functions, little attention has been paid to the triggering of MCP-1 production following FcγR cross-linking, since current views have stressed the role of pro-inflammatory cytokines as the main inductors of MCP-1. In fact, there are only two reports relating MCP-1 production and FcγR cross-linking. A report by Marsh *et al*. has shown that MCP-1 production by human monocytes is significantly enhanced by a factor released from lymphocytes stimulated via FcγRIII (14), and another study has described transcriptional up-regulation of MCP-1 mRNA expression in mature blood monocyte-derived culture macrophages exposed to IFN-γ and solid-phase IgG2a, thus suggesting the occurrence of FcγR cross-linking (15). Since we have previously shown in a rat model that FcγR stimulation of rat macrophages represents a relevant mechanism for polymorphonuclear leukocyte recruitment via the induction of the C–X–C chemokine CINC-1 (5), which is the rat homolog of GROα and the murine KC, we hypothesized that a similar mechanism could contribute to the induction of C–C chemokines in human cell lines. In this study we report the production of MCP-1 by cells of the human monocytic line THP-1 stimulated via FcγR with insoluble aggregates of purified human IgG. This production is higher than that observed in response to the combination of IFN-γ and lipopolysaccharide (LPS), which we found to be the most efficient combination of agonists. Since THP-1 cells express FcγR of the FcγRI and FcγRII classes, but not FcγRIII receptors (16), we can rule out the involvement of FcγRIII in the induction of MCP-1 in this cell line. As to the biochemical mechanisms accounting for the induction, the production of MCP-1 seems associated to the activation of NF-κB by a mechanism involving a selective proteolytic degradation of IκB-α, the blockade of which shows high sensitivity to both proteasome inhibitors and salicylate derivatives.

Methods

Reagents and cell cultures

IgG anti-ovalbumin (OVA) antibody were raised in rabbits as described (17). IgG–OVA equivalence IC were made according to classical procedures. Human IgG, the proteasome inhibitor *N*-acetyl-leucinyl-leucinyl-norleucinal (ALLN, calpain I inhibitor, MG-101), LPS and IFN-γ were purchased to Sigma (St Louis, MO). 2-Hydroxy-4-trifluoromethylbenzoic acid (HTB), a salicylate derivative with potent inhibitory effects on NF-κB activation (18,19), was from Uriach Laboratories (Barcelona, Spain). mAb anti-FcγIR (32.2), anti-FcγRII (IV.3) and anti-FcγRIII (3G8) were from Medarex (Annandale, NJ). mAb anti-human TNF-α was from Genzyme Diagnostics (Cambridge, MA). Heat aggregation of IgG was carried out at 65°C for 20 min, and insoluble aggregates (A-IgG) were separated from soluble aggregates and monomeric IgG by centrifugation at 10,000 *g* for 20 min. Heat aggregation provides a mixture of 15–22S complexes as judged from ultracentrifugation assays. The composition of the pool of IgG was checked by fast protein liquid chromatography and found to show an irrelevant composition according to the current distribution of the human subclasses: IgG1 66%, IgG2 22%, IgG3 8% and IgG4 4%. This composition makes a total amount of subclasses binding to FcγR (IgG1 and IgG3) of 74% of all the IgG contained in the aggregates. THP-1 cells were cultured in plastic dishes in RPMI 1640 medium supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, 50 μg/ml gentamicin, 2 mM glutamine and 10% heat-inactivated FCS. The amount of serum was reduced to 2% in the samples used for the assay of MCP-1 protein. Human peripheral monocytes were isolated from blood by density gradient centrifugation in Ficoll solution and adherence to plastic dishes as described (19). Reagents for the detection of MCP-1 by ELISA were from R & D Systems (Minneapolis, MN). Antisera against p65 and p50 members of the NF-κB/Rel family were a kind gift of Dr Nancy Rice (National Cancer Institute, Frederick, MD). Antibodies against c-Rel and anti-IκB-α, IκB-β and IκB-ε were from Santa Cruz Biotechnology (Santa Cruz, CA).

ELISA assay of human MCP-1 production

MCP-1 was assayed in 200 μl supernatant samples from 3×10^6 THP-1 cells. The procedure was carried out according to the manufacturer's instructions using a specific mouse monoclonal anti-human MCP-1 antibody as the capture antibody and a polyclonal rabbit anti-human MCP-1 antibody conjugated to horseradish peroxidase as the detection antibody. The ELISA was developed using a peroxidase reaction and the standard curve for this ELISA was constructed with recombinant human MCP-1. The minimum detectable dose of MCP-1 of this assay is 5 pg/ml.

Western blot analysis of NF-κB/Rel and IκB proteins

Cells were washed with ice-cold PBS, and lysed in 0.1 ml of ice-cold extraction buffer containing 10 mM HEPES, 2 mM EGTA, 10% glycerol, 10 μg/ml leupeptin, and 1 mM PMSF, pH 7.4. Cell lysate was mixed with Laemmli's buffer and boiled for 5 min. The amount of protein in each sample was assayed using the Bradford reagent and equal amounts of protein were loaded on each lane of a 10% SDS—PAGE gel. Proteins were transferred to nitrocellulose membranes using a semidry transfer module. The membranes were blocked with BSA for 2 h, washed with Tris-buffered saline containing Tween 20 and used for immunoblotting using anti-IκB antibodies, followed by donkey anti-rabbit IgG–horseradish peroxidase antibody. Detection was performed using the Amersham (Little Chalfont, UK) ECL system.

Electrophoretic mobility shift assay

THP-1 cells were washed with ice-cold hypotonic lysis buffer (10 mM HEPES–KOH, pH 7.9, 10 mM KCl, 1.5 mM $MgCl_2$, 0.5 mM DTT, 0.5 mM PMSF, 5 μg/ml aprotinin, 5 μg/ml leupeptin and 0.6% Nonidet P-40). The cells were allowed to swell on ice for 10 min and vortexed vigorously for 10 s. Unbroken cells were eliminated by centrifugation at 1000 *g* for 10 min and the nuclei were collected by centrifugation at 15,000 *g* for 1 min in a microcentrifuge. The nuclear pellet was resuspended in high salt extraction buffer containing 25% glycerol and 0.5 M KCl, and the nuclear extract was obtained by pelleting for 30 min at 105,000 *g* in an Optima TL ultracentrifuge (Beckman, Palo Alto, CA). Twenty-two-mer double-stranded oligonucleotide probes containing NF-κB were end-labeled with [γ-^{32}P]ATP using T4 polynucleotide kinase and separated from the unincorporated label by minic-olumn chromatography. The κB sequence used was, sense 5′-AGTTCAGGGGAATTTCCCAGGC-3′ and the complement 5′-GCCTGGGAAATTCCCCTGAACT-3′. Then 10 μg of nuclear protein was incubated for 20 min on ice with radiolabeled oligonucleotide probes (2–6×10^4 c.p.m.) in a 25 μl reaction buffer containing 2 μg of poly(dI–dC), 10 mM Tris–HCl, pH 7.5, 100 mM NaCl, 1 mM EDTA, 1 mM DTT, 8% Ficoll and 4% glycerol. Nucleoprotein–oligonucleotide complexes were resolved by electrophoresis in a 4% non-denaturing polyacrylamide gel in Tris–borate–EDTA electrophoresis buffer at 175 V for 3 h at 4°C. The gel was dried and autoradiographed with an intensifying screen at –80°C for 2–12 h. The specificity of the DNA–protein complex was confirmed by competition with a 100-fold molar excess of unlabeled nucleotide containing the consensus sequence.

Results

Insoluble aggregates of human IgG stimulate MCP-1 production by THP-1 cells

Incubation of THP-1 cells with concentrations of A-IgG >50 μg/ml induced a dose-dependent production of MCP-1 as judged from the presence of detectable protein in the cell culture supernatants. Plateau values were reached at 200–300 μg/ml (Fig. 1A). MCP-1 production was time dependent and increased for at least 28 h after the addition of the stimulus (Fig. 1B). The production of MCP-1 observed under these conditions was compared with that elicited by other agonists acting on THP-1 cells. As shown in Fig. 2(A), both IFN-γ and LPS alone induced a low production of MCP-1. In contrast, combination of both agonists significantly increased the production, although below the level produced by 100 μg/ml of A-IgG. However, the production of MCP-1 elicited by combination of IFN-γ and LPS was more rapid than that elicited by A-IgG, as judged from the detection of plateau values after 4 h of incubation. Experiments using as agonist monomers of IgG obtained from solutions of IgG subjected to ultracentrifugation for 1 h at 105,000 *g* to sediment the aggregates that could be spontaneously formed showed no MCP-1 production, thus suggesting that FcγR receptors should be cross-linked to activate THP-1 cells. Further evidence of the involvement of FcγR receptors was obtained from the use of IgG–OVA equivalence IC, that produced a dose-dependent production of MCP-1 (Fig. 2B), which was not produced by either isolated OVA (up to 100 μg/ml) or antibody, nor it could be attributed to endotoxin contamination since this was not influenced by the addition of 10 μg/ml polymyxin B. Attempts to characterize the subtypes of FcγR involved in MCP-1 production were carried out with anti-FcγR mAb and

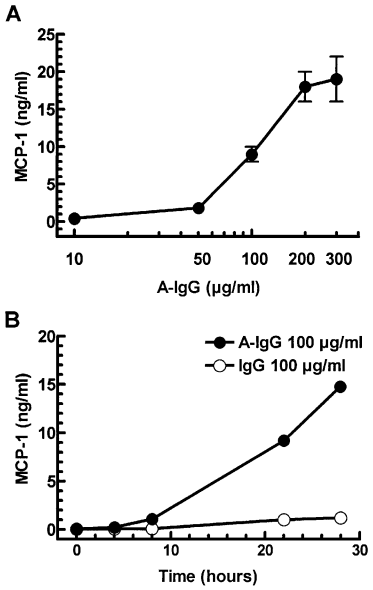

Fig. 1. Production of MCP-1 by THP-1 cells. Cells were stimulated with different concentrations of A-IgG for 24 h. At the end of this period the medium was collected for assay of MCP-1 protein by an ELISA assay. Data represent mean ± SEM of three experiments in duplicate (A). A time-course experiment with 100 μg/ml A-IgG is shown in (B).

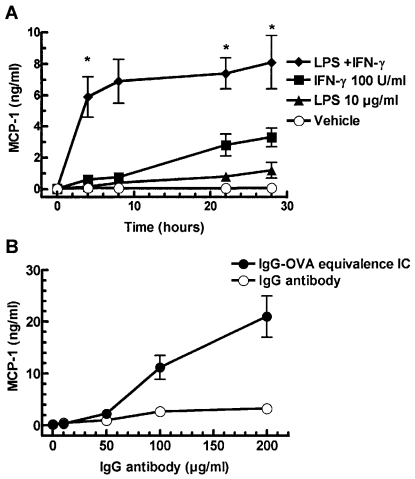

Fig. 2. Production of MCP-1 by THP-1 cells stimulated with different stimuli. Cells were stimulated with LPS and IFN-γ, and the production of MCP-1 assayed in the cell culture medium at the times indicated. Data represent mean ± SEM of four experiments in duplicate. $^{*}P <$ 0.05 as compared to the production elicited by each agonist alone (A). The production of MCP-1 by different amounts of IgG–OVA equivalence IC and the effect of the same amount of rabbit IgG antibody is shown in (B). Data represent mean ± SEM of three experiments in duplicate.

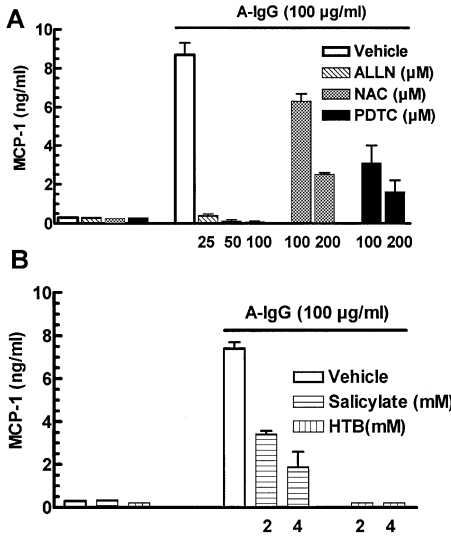

Fig. 3. Pharmacological modulation of the production of MCP-1 elicited by A-IgG in THP-1 cells. The additions were added 1 h prior to the stimulation by A-IgG. Data represent mean ± SEM of four to six independent experiments in duplicate.

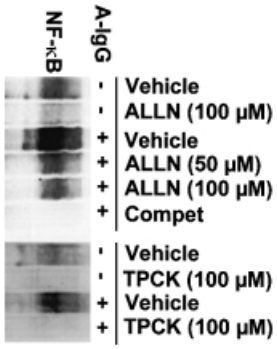

Fig. 4. Effect of the proteasome inhibitor ALLN and the protease inhibitor TPCK on the activation of NF-κB elicited by A-IgG. THP-1 cells were incubated with the indicated additions in the presence and absence of A-IgG for 2 h, and then the cell lysate was taken for the assay of κB-binding activity in the nuclear extract.

a goat anti-mouse secondary antibody using an experimental procedure designed from previous reports (20–22). For this purpose 5×10^6 THP-1 cells were incubated at 4°C for 30 min with 5 μg/ml of each mAb (32.2, IV.3 and 3G8). At the end of this period 10 μg/ml of the secondary antibody were added and the cells were left overnight at 37°C. The amount of MCP-1 obtained under these conditions was <0.134 ng/ml in two independent experiments in duplicate, thus suggesting that Fc–FcγR interactions could be necessary to induce release of MCP-1, as it has been reported for TNF-α production by human monocytes (20).

Since both NF-κB is involved in the up-regulation of MCP-1 in a number of cell systems and FcγR cross-linking elicits NF-κB activation in macrophagic cells (23), we hypothesized that this transcription factor could be involved in the regulation of MCP-1 production in THP-1 cells. To address this issue, we used a series of chemically unrelated compounds which share the common property of inhibiting NF-κB activation, e.g. the proteasome inhibitor ALLN (24), the antioxidant pyrrolidine dithiocarbamate (PDTC), and *N*-acetyl-cysteine (NAC), sodium salicylate and its derivative the compound HTB (18). As shown in Fig. 3(A and B), both ALLN and HTB elicited a complete inhibition of MCP-1 production, whereas NAC, PDTC and sodium salicylate behaved as less potent inhibitors. Further attempts to relate the effect of these compounds on MCP-1 induction with NF-κB activation were addressed by assessing their effect on the translocation of κB-binding proteins to the nucleus. As shown in Fig. 4, 100 μM ALLN inhibited NF-κB activation by A-IgG to the level of binding activity observed in resting cells, thus being consistent with the involvement of κB binding sites in the regulation of MCP-1 production elicited by triggering FcγR. A similar inhibitory effect was produced by the protease inhibitor *N*-tosyl-phenylalanyl-chloromethylketone (TPCK), which also interferes with the activation of NF-κB by blunting the proteolytic degradation of IκB.

The possible existence of an autocrine loop involving TNF-α in the production of MCP-1 elicited by FcγR cross-linking was addressed by performing some experiments in the presence of 100 μg/ml anti-TNF-α. Stimulation of THP-1 cells by A-IgG in the presence of anti-TNF-α produced a small decrease of MCP-1 production (6.7 ± 0.7 versus 8.7 ± 1 ng/ml in control cells, $n = 6$), which did not reach statistical significance.

Attempts to address the biological relevance of these findings were carried out in adhered human monocytes. Monocyte monolayers stimulated with 100 μg/ml A-IgG for 24 h produced 3.5 ± 1.6 ng/ml MCP-1 versus 0.1 ± 0.01 ng/ml in control cells ($n = 3$), thus indicating that the results observed in the monocytic THP-1 cell line can be relevant for pathophysiological conditions.

A-IgG stimulate NF-κB activation in THP-1 cells

Stimulation of THP-1 cells with A-IgG induced a time- and dose-dependent activation of NF-κB as judged from the appearance of κB-binding activity in the nuclear extracts of cells obtained for at least 8 h after the addition of the stimulus (Fig. 5A). This activation was dose dependent, since it was slightly detectable at a concentration of 25 μg/ml and increased up to 200 μg/ml (Fig. 5B). This is a relevant property of this monocytic/macrophagic lineage, since many cell lines of this type, as well as B cells, show constitutive nuclear NF-κB activity, thus precluding a straightforward assessment of the role of this transcription factor following cell stimulation (25). Characterization of the complexes with antibodies raised against NF-κB/Rel proteins showed the involvement of p65 and p50, but not c-Rel protein (Fig. 6). The involvement of p65 was further confirmed by Western blot of nuclear extract with anti-p65 antibodies, which showed a significant increase of the protein 1 h after stimulation, but not prior to the addition of the stimulus (Fig. 7A). Attempts to characterize IκB proteins involved in FcγR signaling were carried out by immunoblot of cell lysate with specific antibodies. As shown in Fig. 8(A), IκB-α protein disappeared from the lysate 15 min after stimulation and increased again to reach pre-stimulation levels at 2–4 h. Interestingly, when THP-1 cells were incubated with A-IgG in the presence of 2 mM HTB, i.e. a dose that produces a complete inhibition of MCP-1 production, IκB-α degradation

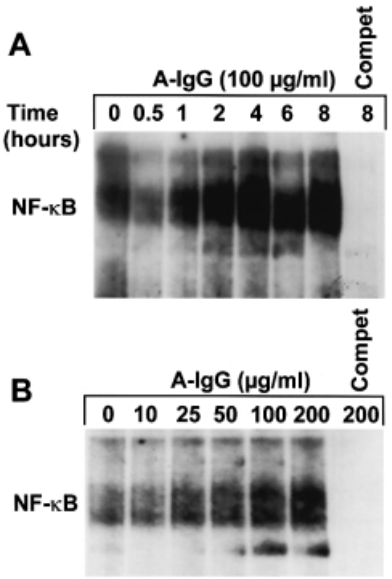

Fig. 5. Activation of NF-κB by A-IgG in THP-1 cells. Lysates from cells incubated for different periods with A-IgG were used for the assay of κB-binding activity (A). The effect of an incubation for 2 h in the presence of different concentrations of A-IgG is shown in (B). The lanes marked 'Compet' indicate that the binding reaction was conducted in the presence of a 100-fold molar excess of unlabeled nucleotide containing the consensus sequence.

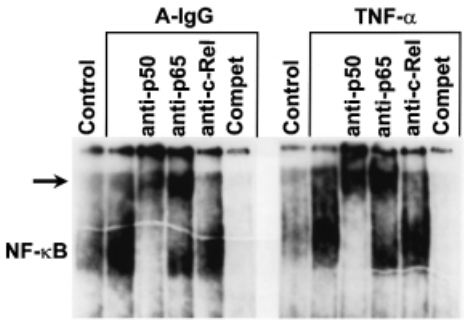

Fig. 6. Characterization of NF-κB/Rel proteins in the κB-binding complexes. The nuclear extracts were obtained from THP-1 cells stimulated with 100 μg/ml A-IgG or 100 U/ml TNF-α for 2 h and used for an incubation for 15 min at 4°C with a 1:40 dilution of the indicated polyclonal rabbit antibodies prior to the addition of the ^{32}P-labeled oligonucleotide probe. The protein–oligonucleotide complexes supershifted by the antibodies are noted by an arrow. The lanes marked 'Compet' indicate that the nuclear extracts were incubated with the ^{32}P-labeled probe in the presence of a 100-fold excess of unlabeled probe.

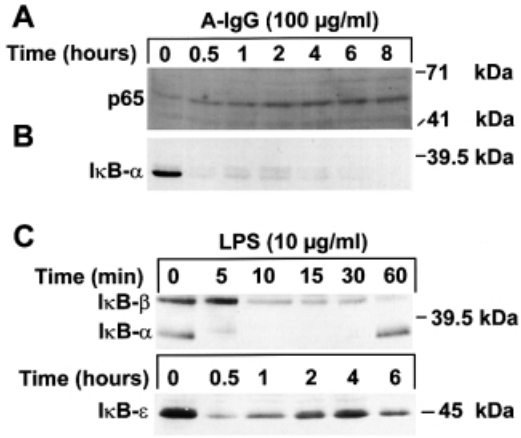

Fig. 7. Translocation of p65 NF-κB/Rel protein to the nucleus and degradation of IκB-α protein in THP-1 cells stimulated with A-IgG. Nuclear extracts from cells incubated with 100 μg/ml A-IgG for the times indicated were subjected to SDS–PAGE and immunodetection of p65 NF-κB/Rel protein using the Amersham ECL system (A). In another experiment, cells were stimulated with A-IgG in the presence of 10 μg/ml cycloheximide and the cell lysate used for the immunodetection of IκB-α protein (B). The effect of 10 μg/ml LPS on IκB degradation is shown in (C). The blot showing Iκ-α and Iκ-β was carried out by incubating the membrane simultaneously with both anti-Iκ-α and anti-Iκ-β antibody.

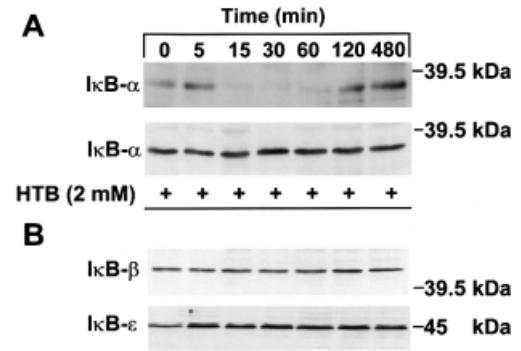

Fig. 8. Immunodetection of IκB proteins in THP-1 cells stimulated with A-IgG. Cells were stimulated with 100 μg/ml A-IgG for the times indicated and at the end of these periods the cell lysate was used for SDS–PAGE and immunodetection of IκB-α (A), IκB-β and IκB-ε (B). The lower panel on (A) represents an experiment conducted in cells preincubated with 2 mM HTB for 1 h prior to the addition of A-IgG.

was not observed (Fig. 8A, lower panel), thus suggesting that breakdown of this protein may play a role in MCP-1 production in THP-1 cells. In a parallel experiment conducted in cells treated with the protein synthesis inhibitor cycloheximide at the concentration of 10 μg/ml, resynthesis of IκB-α was abrogated (Fig. 7B), thus agreeing with current views on the regulation of NF-κB activation, which stress its dependence on the synthesis of new proteins (26). In contrast, IκB-β and IκB-ε expression did not change during this period of time (Fig. 8B), thus suggesting that the activation of NF-κB by FcγR cross-linking involves selective proteolysis of IκB-α and differs from the effect of LPS, which also produces degradation of IκB-β and IκB-ε (Fig. 7C).

Discussion

In this study we have addressed the effect of FcγR cross-linking on the induction of the C–C chemokine MCP-1 in a human cell line of the monocytic/macrophagic lineage and have attempted the characterization of the biochemical mechanism involved in this induction. Since the data is consistent with the results obtained in experiments carried out in primary human monocytes, our findings might have biological relev-

ance on the basis of two different reasons: (i) FcγR cross-linking has been depicted as a central event in tissue injury (1,4,27), and (ii) MCP-1 attracts monocytes, $CD45RO^+$ T lymphocytes and NK cells *in vitro* with an EC_{50} ~ 500 pM (28,29), thus making likely the involvement of this chemokine in diseases that are characterized by mononuclear cell infiltrates such as atherosclerosis, multiple sclerosis or rheumatoid arthritis, since high levels of MCP-1 expression have been reported at inflammatory sites in these clinical settings (10,30,31). Expression of MCP-1 is regulated at the transcriptional level, and shows both stimulus-specific and tissue-specific regulation, although in most systems proinflammatory cytokines and IFN-γ are the most effective inducers. Accordingly, κB sites (32), TRE (12) and GAS sites (13) are the most relevant regulatory sequences involved in MCP-1 regulation. Our attempts to depict the regulatory elements involved in MCP-1 induction by FcγR cross-linking first pointed to NF-κB in view of the previous description of the triggering of this transcription factor after occupancy of FcγR, and its functional relevance in the regulation of both CINC-1 and the inducible isoform of the NO synthase (23). In the present study we have found a time- and dose-dependent activation of NF-κB in response to FcγR occupancy, the translocation to the nucleus of NF-κB/Rel proteins with a composition indistinguishable from that of the complexes produced in response to TNF-α and a blunting of MCP-1 production by inhibitors of NF-κB activation. Taken together, these results suggest the involvement of the κB site in the transcriptional regulation of MCP-1, although demonstration of this hypothesis would require transfection studies with the MCP-1 promoter.

Since cross-linking of FcγR induces secretion of TNF-α by both human monocytes (20) and murine macrophages (22), it seems likely that TNF-α could be involved in an autocrine loop enhancing the MCP-1 production. In fact, studies in rat macrophages stimulated with IC have shown a partial inhibition of MCP-1 production in the presence of an anti-TNF-α antibody (33). Our data using a similar approach does not rule out a possible contribution of this mechanism in our system, although the diminution of MCP-1 production observed in cells stimulated in the presence of anti-TNF-α antibody did not reach statistical significance.

Our attempts to identify the IκB proteins engaged in FcγR signaling showed degradation of IκB-α, whereas both IκB-β and IκB-ε displayed a steady level of expression during MCP-1 induction, thus suggesting that they are not involved in the process of proteolytic cleavage of the inhibitor proteins required for the translocation of NF-κB to the nucleus. This point deserves a detailed discussion since both IκB-β and IκB-ε show a high level of expression in THP-1 cells and are degraded in response to LPS with a time-course parallel to that of IκB-α (34). Moreover, IκB-ε associates to a subset of p65/cRel NF-κB complexes involved in the activation of chemokines in some cell types, e.g. IL-8 (35) and MCP-1 (36). Taken together, our data show a distinct pathway for NF-κB activation in THP-1 cells following cross-linking of FcγR, which involves degradation of IκB-α and formation of p50/p65 NF-κB/Rel complexes.

The elevated production of MCP-1 induced by A-IgG as compared to phorbol ester (not shown), LPS and IFN-γ points to a cell-specific regulation of MCP-1 production in THP-1 cells linked to the *trans*-activation of κB sites, and to a limited functional relevance of TRE and GAS sites, which are respectively activated by phorbol ester and IFN-γ, whereas these regulatory elements could be of physiological relevance in other cell types. Moreover, the results observed with pharmacological inhibitors of NF-κB also point to the functional involvement of this transcription factor since both the expression of MCP-1 and the nuclear translocation of the factor were similarly affected by ALLN, HTB, PDTC and TPCK. The effect of the proteasome inhibitor ALLN deserves detailed attention. In fact, there is general agreement that this type of compound blocks the nuclear translocation of NF-κB and affects the expression of genes which are under the transcriptional regulation of κB sites, e.g. adhesion molecules (37), IL-8 (38) and cyclooxygenase-2 (39). However, the selectivity of the proteasome inhibitors has been challenged, since it has been proposed that they block MCP-1 expression by a mechanism independent of IκB-α degradation and the ensuing blockade of NF-κB translocation (40,41). Since these studies have been carried out in endothelial cells stimulated with IL-1β, a likely explanation for this difference could be that proteasome inhibitors could exert additional pharmacological effects on the functional activity of the NF-κB/Rel proteins, that are of particular relevance in the response to particular agonists.

As to the subclasses of FcγR involved in the response to A-IgG, our data do not allow us to distinguish between FcγRI and FcγRII, since we have been unable to disclose MCP-1 production by cross-linking FcγR with mAb, thus pointing to the requirement of high-affinity Fc–FcγR interactions, as it has been reported for TNF-α production by human monocytes (20). Moreover, since a central role for FcγRIII has been proposed in immune effector functions in murine macrophages (42) and even a marginal contribution of FcγRI in FcγRIII-deficient animals (43), a straightforward answer to this point can only be provided by transfection experiments with cDNA encoding for specific receptors. In summary, this study has shown a prominent effect of FcγR cross-linking on the up-regulation of the expression of MCP-1. This effect seems dependent on the activation of NF-κB linked to the proteolytic degradation of IκB-α, and involves at least both p50 and p65 NF-κB/Rel proteins. Attempts to modify MCP-1 expression by pharmacological means have shown remarkable sensitivity to conventional inhibitors of NF-κB activation, thus highlighting the clinical relevance of NF-κB inhibitors in immune-mediated tissue injury.

Acknowledgements

Dr Nancy Rice from National Cancer Institute (Frederick, MD) is thanked for the gift of anti-p50 and anti-p65 antibodies. Dr Manuel Merlos from Uriach SA is thanked for the gift of HTB. This study has been supported by grants from Plan Nacional de Salud y Farmacia (grants SAF98-0176 and 1FD97-0590).

Abbreviations

A-IgG	insoluble aggregates of human IgG
ALLN	*N*-acetyl-leucinyl-leucinyl-norleucinal
FcγR	receptor for the Fc portion of IgG
GAS	IFN-α-activated site

HTB	2-hydroxy-4-trifluoromethylbenzoic acid
IκB	inhibitor of NF-κB activity
IC	immune complex
LPS	lipopolysaccharide
NAC	*N*-acetyl-cysteine
OVA	ovalbumin
PDTC	pyrrolidine dithiocarbamate
TNF	tumor necrosis factor
TPCK	*N*-tosyl-phenylalanine chloromethyl ketone
TRE	12-α-tetradecanoylphorbol 13-acetate-response element

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