Diminished Expression of Transcription Factors Nuclear Factor κB and CCAAT/Enhancer Binding Protein Underlies a Novel Tumor Evasion Mechanism Affecting Macrophages of Mammary Tumor–Bearing Mice

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Abstract

Interactions between malignant tumors and the host immune system shape the course of cancer progression. The molecular basis of such interactions is the subject of immense interest. Proinflammatory cytokines produced by macrophages are critical mediators of immune responses that contribute to the control of the advancement of neoplasia. We have shown that the expressions of interleukin 12 (IL-12) and inducible nitric oxide synthase (iNOS) are decreased in macrophages from mammary tumor–bearing mice. In this study, we investigated the causes of IL-12 dysregulation and found deficient nuclear factor κB (NFκB) and CCAAT/enhancer binding protein (C/EBP) expression and function in tumor bearers' peritoneal macrophages. The constitutive expressions of NFκB p50, c-rel, p65, and C/EBPα and β, as well as the lipopolysaccharide-induced nuclear translocation and DNA binding of NFκB components and C/EBPα and β, are profoundly impaired in macrophages from mice bearing D1-DMBA-3 tumors. Because similar findings occur with the *iNOS* gene, it seems that it represents a novel mechanism by which tumor-derived factors interfere with the host immune defenses. (Cancer Res 2005; 65(22): 10578-84)

Introduction

Persistent or chronic inflammation in a tissue or an organ contributes to tumorigenesis (1). However, once a tumor is generated and becomes established, its progression is associated with increasing levels of immunosuppression in the host regardless of tumor location or etiology, both in laboratory animals and in humans (2–5). One of the diverse mechanisms by which established tumors succeed to escape from the immune defenses and induce a depressed immune status in the host is by disturbing the delicate equilibrium of cytokine expression patterns that regulate immune function in the healthy host (6–8). Proinflammatory cytokines, produced mainly by macrophages and dendritic cells, are critical in the development of the initial inflammatory response as well as in the subsequent immune adaptive response (9).

Previous work from our laboratory has shown that, on stimulation with lipopolysaccharide (LPS), thioglycollate-induced peritoneal macrophages (PEM) isolated from BALB/c mice bearing the D1-DMBA-3 mammary tumor display depressed innate immune functions, characterized by diminished macrophage cytotoxicity, low levels of nitric oxide expression, and decreased production of interleukin (IL)-12 (10–13). In earlier publications, we have shown that the down-regulated production of both IL-12 and inducible nitric oxide synthase (iNOS) is associated with the action of several factors secreted by the mammary tumor used in our studies (12–15). In the present investigation, we analyzed the underlying molecular mechanism whereby this progressive tumor affects the activation of IL-12 genes in macrophages. Deficient nuclear factor κB (NFκB) and CCAAT/enhancer binding protein (C/EBP) expressions, as well as diminished nuclear translocation and DNA binding activities of these transcription factors, were observed in host macrophages. Because similar findings have been noted in studies of the *iNOS* gene promoter of tumor bearers' macrophages (16), these functional dysregulations may represent a manifestation of a more general phenomenon by which tumor-derived factors contribute to tumor evasion by interfering with the expression and function of key transcription factors.

Materials and Methods

Animals and tumors. Male and female BALB/c mice of 10 to 14 weeks of age, maintained by brother-sister mating in our facilities, were used. In all experiments, the mice were age and sex matched. The D1-DMBA-3 mammary adenocarcinoma is a transplantable tumor derived from a non-viral, non-carcinogen-induced preneoplastic alveolar nodule in a BALB/c mouse treated with 7,12-dimethylbenzanthracene (17). The immunogenic tumor is routinely transplanted in BALB/c mice by s.c. injection of 1×10^6 tumor cells. Our institutional animal care and use committee approved the animal experiments.

Macrophage collection and culture. Normal mice and 4-week tumor-bearing mice were injected i.p. with 1.5 mL of 3% thioglycollate. On day 4, the peritoneal exudate cells were obtained by peritoneal lavage with 20 mL of ice-cold RPMI 1640; PEM from normal animals (N-PEM) and tumor bearers (T-PEM) were later purified and cultured as previously described (18).

Reagents. RPMI 1640 containing 10% FCS, 2 mmol/L L-gluthamine, and 100 units of penicillin and 100 μg/mL of streptomycin (all from Hyclone, Logan, UT) was used as culture medium in all experiments. LPS from *Escherichia coli* 055:B5 was purchased from Sigma-Aldrich (St. Louis, MO).

Cytokine ELISA. PharMingen (San Diego, CA) OptEIA sets for mouse IL-12p70 heterodimer, mouse IL-12p40 monomer, and mouse IL-10 were used to measure the amounts of IL-12p70, IL-12p40, and IL-10 proteins in the supernatants of PEM according to the instructions of the manufacturer.

Northern blot. Northern hybridizations were done as previously reported (11) with the following modifications: Total RNA was isolated using RNAwiz reagent (Ambion, Austin, TX) according to the instructions

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of the manufacturer and RNA samples (15 μg) were electrophoresed, transferred, and hybridized using the NorthernMax-Gly set, a glyoxal-based system for Northern blots from Ambion, following the instructions of the manufacturer. The cDNA probes for *IL-12p40* and *p35* genes, a generous gift from Dr. Giorgio Trinchieri (Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, MD), were radiolabeled with [α-^{32}P]dATP (3,000 Ci/mmol; New England Nuclear/Perkin Elmer, Boston, MA) by random primer labeling (Prime-a-Gene, Promega, Madison, WI). β-Actin levels were similarly examined using β-actin cDNA (Clontech Laboratories, Inc., Palo Alto, CA) and served as controls to normalize RNA quantity.

Electrophoretic mobility shift assay. Isolation of nuclear extracts, sequences, preparation of oligonucleotide probes, electrophoretic mobility shift assay (EMSA), and supershift assays were conducted as previously described (15, 16); all antibodies used were from Santa Cruz Biotechnology (Santa Cruz, CA).

Western blot. Whole-cell and nuclear extracts from N-PEM and T-PEM were used. To isolate whole-cell extracts, PEM were lysed using cold radioimmunoprecipitation assay buffer supplemented with protease inhibitor cocktail (Roche, Indianapolis, IN) and sodium vanadate (Roche, 1 mmol/L final concentration) as described in ref. 11. Nuclear extracts were isolated as described in ref. 15. Protein concentration was determined using the BCA Protein Assay Kit (Pierce, Rockford, IL) and Western blots were done as previously described (11). The primary antibodies used were rabbit anti-mouse polyclonals from Santa Cruz Biotechnology, except for IκB kinase (IKK)-β (mouse monoclonal from Imgenex, San Diego, CA) and for the phosphorylated form of IκBα (mouse monoclonal antibody from Santa Cruz Biotechnology); the presence of actin was detected by the use of a rabbit anti-mouse polyclonal antibody (Sigma-Aldrich). For the mouse monoclonal antibodies used, secondary antibodies were goat anti-mouse isotype matched to the corresponding primary antibodies.

Results

Both protein and mRNA levels of proinflammatory molecules are decreased in peritoneal macrophages from tumor bearers. LPS-activated T-PEM from 4-week tumor bearers exhibit an impaired capacity to produce the IL-12p70 heterodimer as measured by ELISA compared with the IL-12 expression in N-PEM (Fig. 1*A*). Because IL-12 is composed of two chains, p40 and p35, we sought to determine whether the IL-12p40 protein synthesis/secretion was also affected in these cells (there is no available antibody to study p35 protein expression). IL-12p40–specific ELISAs were done using 24-hour supernatants of macrophages from normal or 4-week tumor-bearing mice cultured alone or with LPS. As seen in Fig. 1*B*, LPS-activated T-PEM exhibited a down-regulated IL-12p40 monomer production compared with similarly activated cells from normal animals, accounting, at least in part, for the decreased production of IL-12p70 in these cells. It was observed that the levels of IL-12p40 were higher than the levels of IL-12p70 produced by both cell types, as expected (19). Our previous studies have shown that the protein levels of iNOS in T-PEM stimulated with LPS are also down-regulated (11).

To analyze whether IL-12p40 and p35 mRNA expressions were affected in T-PEM, Northern blot experiments were done. The results of these Northern hybridizations (Fig. 1*C*) showed a substantial decrease in the expression of the *p40* gene, together with a slightly diminished *p35* gene expression in LPS-activated T-PEM, as compared with similarly activated N-PEM. Furthermore, no alternatively spliced or aberrant mRNAs were observed in T-PEM for either gene (i.e., only the expected transcripts of 2.3 kb for p40 and 1.3 kb for p35 were detected). As was the case with the protein, we have also previously shown that the iNOS mRNA levels are decreased in T-PEM as compared with the levels in N-PEM (11).

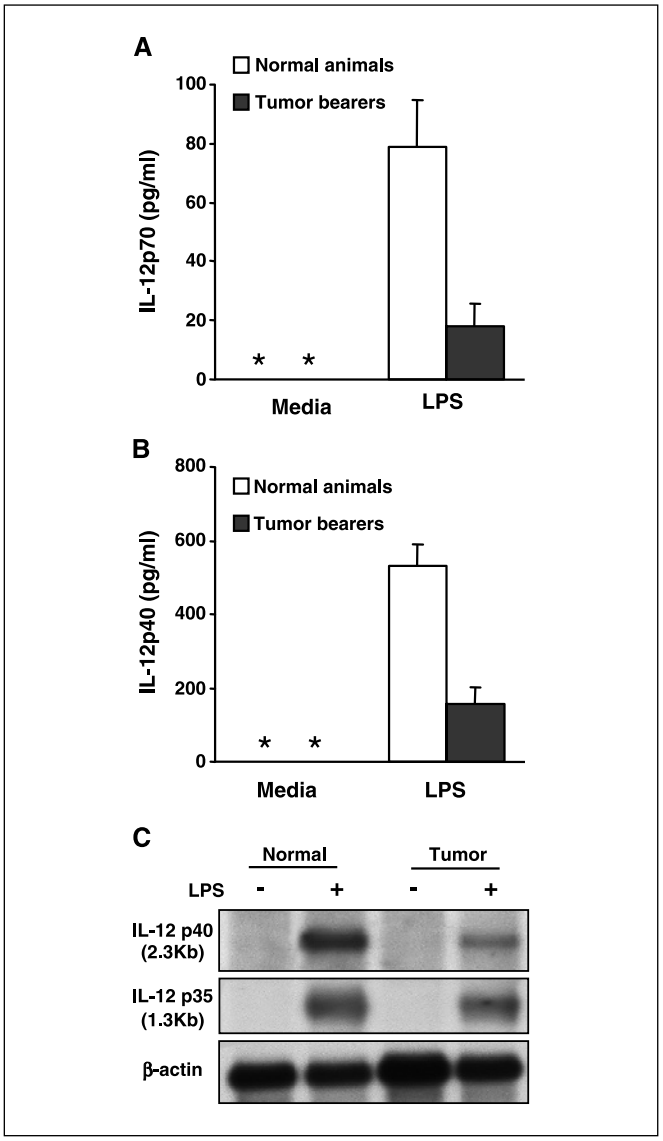

Figure 1. IL-12p40 is down-regulated in macrophages from tumor bearers at the protein and mRNA levels. ELISAs of IL-12p70 (*A*) and IL-12p40 (*B*) measured protein concentration in 24-hour supernatants of macrophages from normal and 4-week tumor-bearing mice cultured with and without LPS (10 μg/mL). *Columns,* mean of four different experiments with similar results; *bars,* SD. *C,* Northern blot of IL-12p40 and IL-12p35 genes in PEM isolated from five normal and five 4-week tumor-bearing mice after culture with and without activation with LPS (10 μg/mL) for 4 hours. Representative of three independent experiments with similar results.

Peritoneal macrophages from tumor bearers exhibit decreased nuclear factor κB and CCAAT/enhancer binding protein bindings to gene promoters of proinflammatory genes. To further analyze the mechanism of the decreased mRNA expression of the IL-12 genes in T-PEM, we studied the functionality of the most relevant transcription factors involved in their expression. Because the *IL-12p40* gene was shown to be the most affected component involved in the IL-12 deficiency in T-PEM, we focused our analysis in this particular gene. The mouse *IL-12p40* gene promoter has been well characterized (20–22) and the transcription factors NFκB and C/EBP are among the major ones involved in its regulation. A study of the binding activities of these two transcription factors to the IL-12p40 promoter was done

by EMSA with nuclear protein extracts isolated from N-PEM and T-PEM, with and without LPS activation, using synthetic double-stranded oligonucleotides matching known sequences of the mentioned transcription factors sites on the mouse *IL-12p40* gene promoter. Our results, using a ^{32}P probe for the NFκB "half site" of the IL-12p40 murine promoter (20), revealed a profound decrease in the binding activity of NFκB in LPS-activated T-PEM (Fig. 2*A*) compared with N-PEM. Competition experiments with cold NFκB and irrelevant Oct-1 binding sites showed the specificity of the binding to the NFκB site. Supershift experiments were done with nuclear extracts of LPS-activated N-PEM and T-PEM to characterize the contribution of the different members of the NFκB transcription factor family in these cells using antibodies against the various NFκB members (p50, p52, p65, c-rel, and relB; Fig. 2*B*). These experiments revealed that p50, p65, and c-rel compose the two major NFκB complexes (designated *a* and *b*, respectively) in PEM as addition of antibodies against these three components caused alterations in the mobility of these complexes. Band *b*, with the increased mobility, represents p50/p50 homodimers whereas the slower band *a* reflects the presence of p50/p65 and p50/c-rel heterodimers. T-PEM exhibit a dramatic decrease in the intensity of band *b*, illustrating a significant reduction of p50/p50 in tumor bearers (Fig. 2*B*). Furthermore, minor participation of relB and p52 can also be observed as expressed by the lower density of the corresponding band patterns after the addition of the respective antibodies. Regarding iNOS, our data, using a ^{32}P probe for the NFκBd unique site from the NFκB consensus sequence (23), revealed a profound decrease in the binding activity of NFκB to the iNOS promoter in LPS-activated T-PEM compared with N-PEM (Fig. 3*A*). Supershift experiments showed, in both N-PEM and T-PEM activated with LPS, that nearly the entire NFκBd complex was shifted in response to anti-p50. Addition of antibodies against p65 and c-rel, respectively, induced a decrease in the intensity of the upper band of the duplex in each case. Antibodies against Rel B or p52 did not affect the mobility or the intensity of the complexes (Fig. 3*B*).

Regarding the C/EBP transcription factor, three major DNA-protein complexes were revealed using a synthetic probe for its binding site on the murine IL-12p40 promoter of N-PEM (21), designated *a, b*, and *c* in Fig. 2*C*. In T-PEM, these binding activities were strongly diminished in both resting and LPS-activated cells. Competition experiments with cold C/EBP and irrelevant Oct-1 binding sites showed a specific binding for the C/EBP species. Supershift analyses were done with the nuclear extracts of LPS-activated N-PEM and T-PEM. Figure 2*D* shows a representative experiment done with antibodies against the different members of this transcription factor family, C/EBPα, β, and δ. The three main DNA-protein complexes formed with the C/EBP synthetic probe correspond to homodimers and heterodimers between C/EBPα, β, and δ (21). Our data revealed a major involvement of α and β and, to a lesser extent, of δ in both cell types. No cooperation between C/EBP with NFκB or activator protein transcription factors was observed in either cell type. Concerning iNOS, we analyzed the binding of nuclear proteins to the NF-IL6 (−153/−142) site of the iNOS promoter (24). Nuclear extracts from T-PEM, as compared with N-PEM, show a significantly diminished binding to the C/EBP sequence in the constitutive as well as the inducible binding after stimulation with LPS (Fig. 3*C*). Supershift experiments revealed, in both N-PEM and T-PEM activated with LPS, that antibodies against C/EBPα and β clearly affected the mobility or the intensity, respectively, of the band complex whereas antibody against C/EBPδ did not induce major changes (Fig. 3*D*)

Diminished binding activities of transcription factors nuclear factor κB and CCAAT/enhancer binding protein are associated with their decreased protein expressions in peritoneal macrophages from tumor bearers. Several reasons

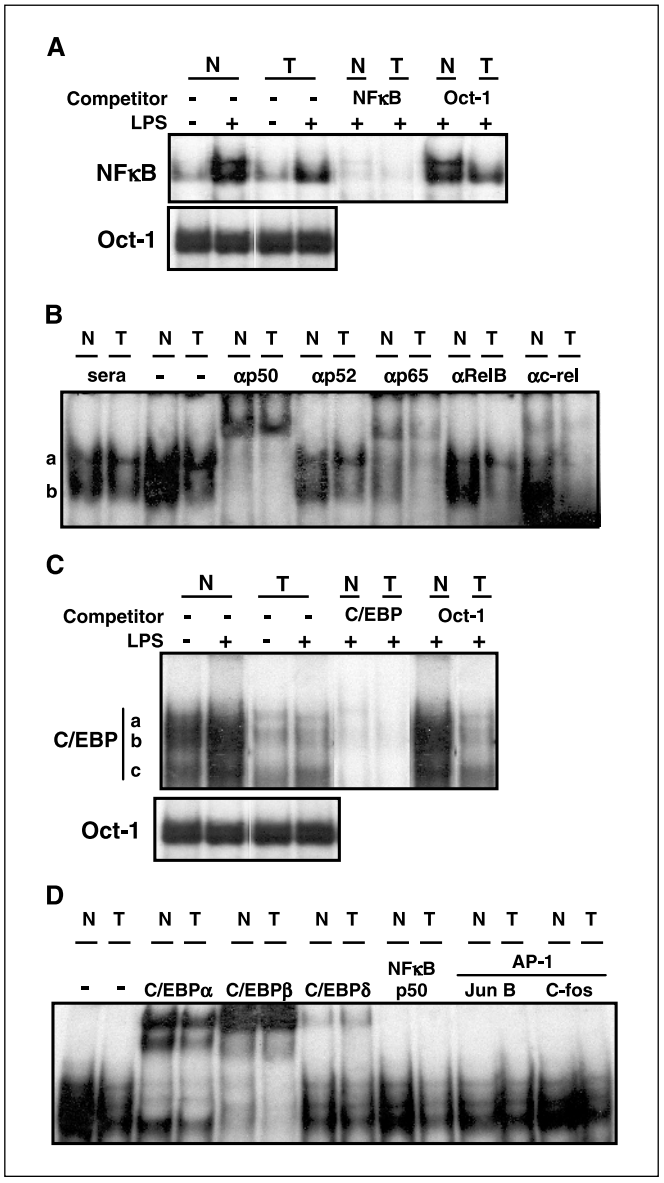

Figure 2. Decreased binding of NFκB and C/EBP to *IL-12p40* gene promoter in 4-week T-PEM. *A,* specific binding of nuclear proteins to an oligonucleotide probe matching the NFκB "half-site" of the IL-12p40 promoter is reduced in T-PEM as assessed by EMSA. Competition with cold NFκB or irrelevant transcription factor Oct-1 showed that the binding was specific for NFκB. Normalization was done when identical amounts of the same nuclear extracts samples were used to bind a radioactive probe of the ubiquitous transcription factor Oct-1, showing equal protein loading in every lane. One of four different experiments with similar results. *B,* identification of the NFκB family proteins binding to the IL-12p40 site with the use of antibodies against p50, p52, p65, relB, and c-rel. Mouse nonimmune sera were used as control. One of four different experiments with comparable results. *C,* specific binding of nuclear proteins to an oligonucleotide probe matching the C/EBP site of the IL-12p40 promoter is reduced in T-PEM as assessed by EMSA. Competition with cold C/EBP or irrelevant transcription factor Oct-1 showed that the binding was specific for C/EBP. One of four different experiments with similar results. *D,* identification of the C/EBP family proteins binding to the IL-12p40 site using antibodies against C/EBPα, β, and δ. Cooperation between C/EBP and NFκB and AP-1 was analyzed using antibodies against NFκB p50, Jun B, and c-fos. One of four different experiments with similar results.

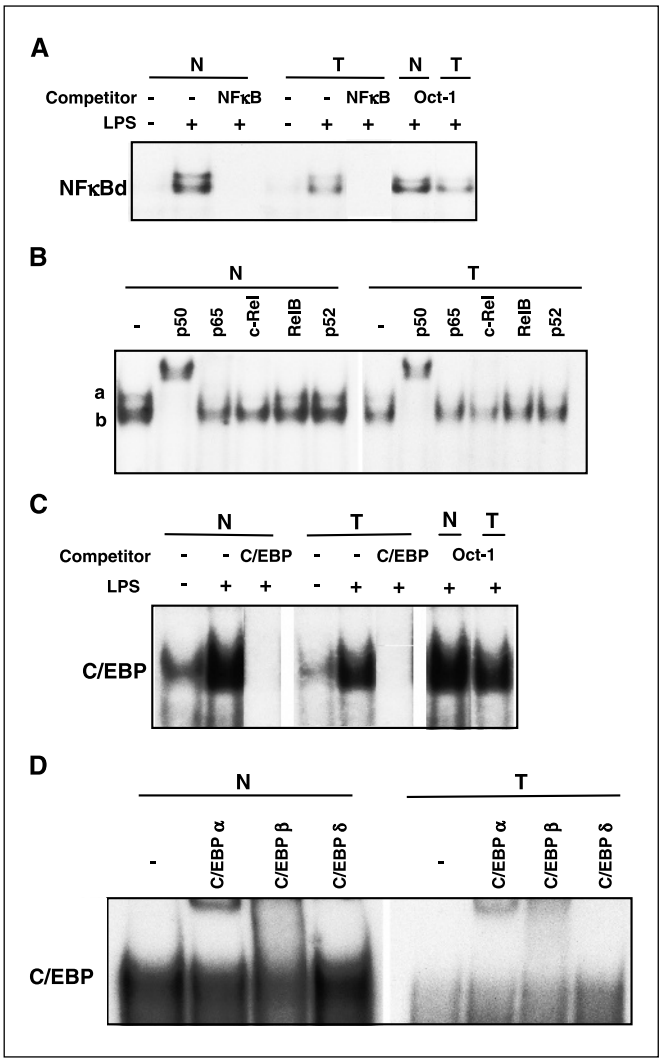

Figure 3. Diminished binding of NFκB and C/EBP to the *iNOS* gene promoter in 4-week T-PEM. *A,* specific binding of nuclear proteins to an oligonucleotide probe matching the NFκBd site of the *iNOS* gene promoter is reduced in T-PEM as assessed by EMSA. Competition with cold NFκB or irrelevant transcription factor Oct-1 showed that the binding was specific for NFκB. One of four different experiments with similar results. *B,* identification of the NFκB family proteins binding to the iNOS site with the use of antibodies against p50, p52, p65, relB, and c-rel. One of four different experiments with comparable results. *C,* specific binding of nuclear proteins to an oligonucleotide probe matching the C/EBP site of the iNOS promoter is reduced in T-PEM as assessed by EMSA. Competition with cold C/EBP or irrelevant transcription factor Oct-1 showed that the binding was specific for C/EBP. One of four different experiments with similar results. *D,* identification of the C/EBP family proteins binding to the iNOS site using antibodies against C/EBPα, β, and δ. One of four different experiments with similar results.

might account for the fact that NFκB and C/EBP isolated from T-PEM bind deficiently to synthetic oligonucleotides representing their actual sites in the IL-12p40 and iNOS promoters. One of them is the existence of diminished amounts of these transcription factors in T-PEM. Using whole-cell extracts isolated from resting N-PEM and T-PEM, we determined by Western blot analysis whether there was a down-regulated protein production of NFκB and C/EBP in T-PEM. Figure 4*A* shows the results of a representative experiment. NFκB c-rel protein is profoundly down-regulated in T-PEM, followed by NFκB p50 and p65 to lesser extents. In addition, C/EBPα and β exhibit decreased expressions in these cells as compared with nonactivated N-PEM.

Peritoneal macrophages from tumor bearers do not exhibit altered amounts of IκBα or its phosphorylated form. Another reason that could account for the decreased binding activity of NFκB in T-PEM is the existence of altered amounts of Iκβα protein or its phosphorylated form in these cells. This possibility was examined by Western blot using whole-cell extracts of resting as well as LPS-activated N-PEM and T-PEM. Iκβα was expressed constitutively in both N-PEM and T-PEM at similar levels (Fig. 4*B*); yet, on LPS activation, its amount decreased significantly to similar levels in both cell types. When the phosphorylated form of IκBα (p-IκBα) was examined, it was observed that both cell types exhibited increased levels of phosphorylated IκBα on LPS activation; however, there were no prominent differences in its expression between N-PEM and T-PEM. Thus, both IκBα and its phosphorylation remain intact in T-PEM and do not explain the down-regulated binding activity of NFκB observed in T-PEM. The presence of IKKα and IKKβ proteins, the two kinase domains of the IKK complex responsible for the IκB phosphorylation (25), was also assessed in both groups of macrophages. As shown in Fig. 4*B*, IKKα was strongly expressed in both N-PEM and T-PEM but was not modulated by LPS in either cell type, and there were no differences in its expression levels between N-PEM and T-PEM. On the other hand, IKKβ protein could be detected neither in N-PEM nor in T-PEM.

Nuclear factor κB and CCAAT/enhancer binding protein nuclear translocations are reduced in peritoneal macrophages from tumor bearers. The diminished binding activities of NFκB and C/EBP to the IL-12p40 and iNOS promoter regions in T-PEM may be also due to their decreased nuclear translocations in these cells. To examine this possibility, we analyzed nuclear extracts from N-PEM and T-PEM, with and without activation with LPS, by Western blotting. Figure 4*C* shows that unstimulated N-PEM show very little NFκB nuclear protein whereas after LPS activation for 1 hour, NFκB p50, p65, and c-rel translocate to the nucleus. However, a dramatic decrease in the amounts of p65, p50, and c-rel proteins can be observed in the nucleus of T-PEM after 1 hour of LPS stimulation, especially in the case of p65. Furthermore, the nuclear translocation of both C/EBPα and β was substantially reduced in T-PEM as compared with N-PEM, especially C/EBPα, although LPS did not modulate C/EBP in either cell type, as was also shown by EMSA.

Although peritoneal macrophages from tumor bearers exhibit a decreased production of interleukin-12, they do not seem to be M2 macrophages. Low IL-12 production accompanied by increased IL-10 expression is associated with macrophages of the M2 phenotype (26). To analyze whether T-PEM isolated from mice bearing advanced (4-week) tumors behave as M2 macrophages, their IL-10 expression levels were studied. Figure 5 shows that the production of IL-10, although somewhat variable, revealed no significant differences between the amounts produced by N-PEM and the levels expressed by T-PEM at various times after tumor implantation.

Discussion

Peritoneal macrophages from mice bearing advanced D1-DMBA-3 mammary tumors are profoundly dysfunctional, and among other defects, they exhibit depressed levels of IL-12 (12) and iNOS (11). An association of these deficiencies with decreased binding activities of the transcription factors NFκB and C/EBP in macrophages from tumor bearers has been documented. Because NFκB

and also C/EBP play central roles in the control of inflammation and immunity, they represent ideal targets for tumors to disrupt immune responses (27). Using animal models and human tumors, Sica et al. (28) have also found a strong association between a decreased production of IL-12 and a diminished binding activity of NFκB in tumor-associated macrophages.

One reason why reduced binding activities of NFκB and C/EBP to the IL-12p40 and iNOS promoters are exhibited by macrophages from tumor bearers is that there is a diminished amount of these transcription factors in these cells. We have consistently observed that nonstimulated T-PEM show decreased protein expressions of NFκB and C/EBP as compared with macrophages from normal mice. Very little is known about the regulation of NFκB and C/EBP expressions because the majority of information published relates to their function. In the studies by Sica et al. (28), these investigators did not evaluate whether there was an effect on the constitutive levels of expression of NFκB in tumor-associated macrophages. Reports of decreased NFκB expression in the context of disease states are very scarce in the literature. Wong et al. (29) showed that interleukin-2-deficient T cells from systemic lupus erythematosus patients are characterized by diminished binding activity of NFκB and decreased expression of the NFκB p65 protein. In a recent study, Kessel et al. (30) have shown that increased $CD8^+$ T-cell apoptosis in systemic sclerosis patients is associated with low levels of NFκB p50. These authors emphasize that the specific mechanisms that lead to the decreased levels of NFκB in these cases are unclear.

Another possible explanation for the decreased binding activity of NFκB in T-PEM could be the existence of altered levels of IκBα, its phosphorylated form, or members of the upstream kinase complex IKK responsible for the phosphorylation of IκBα (31, 32). However, no evidence for dissimilar expression of IκBα or p-IκBα between N-PEM and T-PEM was found. Very recently, Lawrence et al. (33) described a new role for IKKα in the negative regulation of macrophage activation and inflammation, in which IKKα contributes to suppression of NFκB activity by accelerating both the

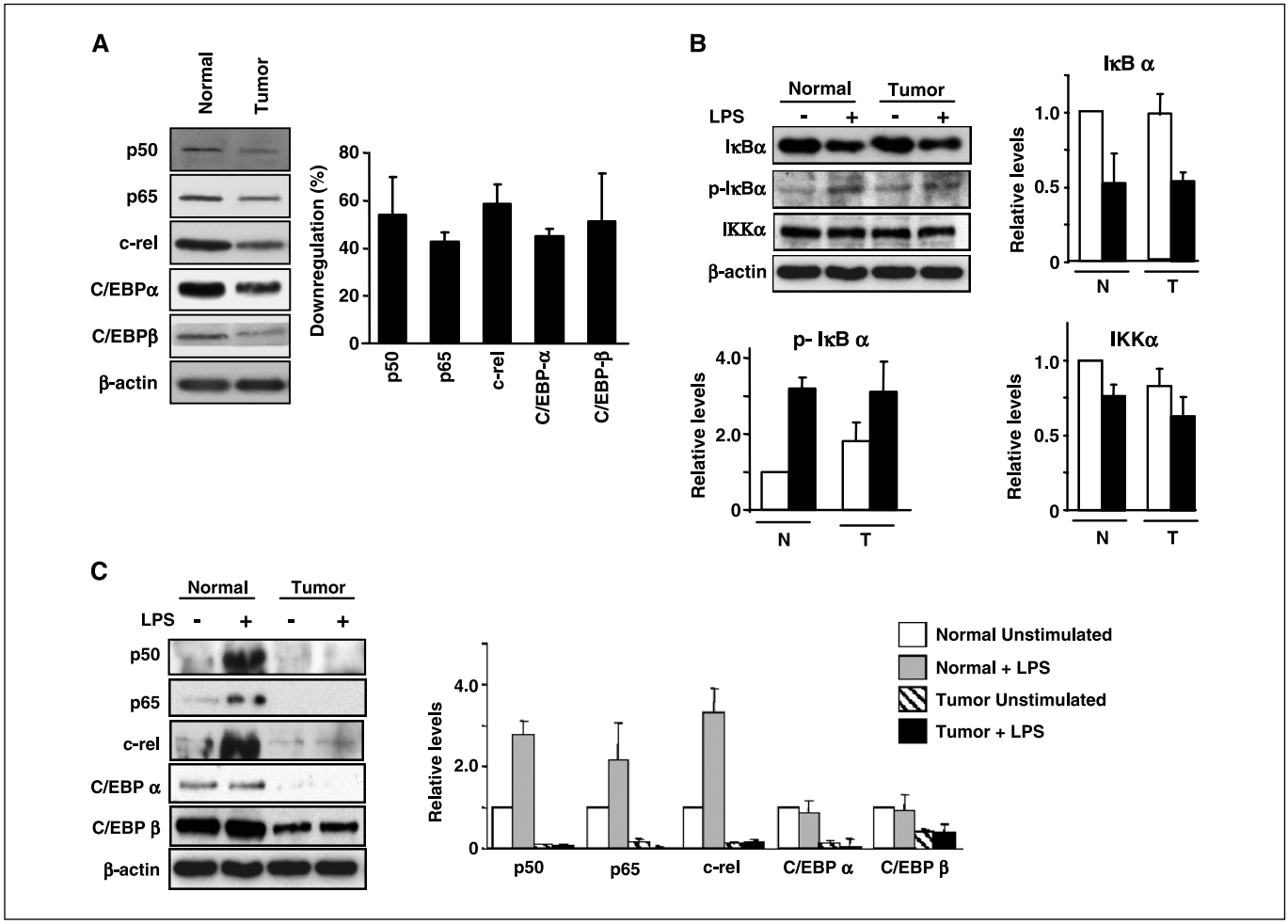

Figure 4. Expression and nuclear translocation of NFκB and C/EBP proteins is diminished in macrophages from tumor bearers. PEM from five normal animals and five 4-week tumor bearers were isolated, pooled, and cultured before obtaining whole-cell extracts or nuclear proteins for Western blot analyses. *A,* whole-cell protein was isolated from resting N-PEM and T-PEM and the levels of the NFκB family members p50, p65, and c-rel, as well as of C/EBPα and β, were investigated. Histogram shows densitometry results expressing percentages of protein expression down-regulation in T-PEM, as compared with N-PEM, after standardization with β-actin. *B,* whole-cell protein was isolated from N-PEM and T-PEM with and without LPS treatment for 1 hour, and the presence of IKKα, IκBα, and p-IκBα was assessed. Histograms show densitometry results expressing relative units standardized with β-actin in which values are referred to unstimulated N-PEM. *C,* nuclear extracts were obtained from N-PEM and T-PEM with and without LPS activation for 1 hour and the nuclear translocation of the various NFκB and C/EBP components was assessed. Histogram expresses relative units standardized with β-actin and values are referred to unstimulated N-PEM. Mouse β-actin was used to assess that equal amounts of protein were loaded in the gels. In all the cases, results are from representative experiment of four different ones with similar outcome. *Columns,* mean of the data obtained from the different experiments; *bars,* SD.

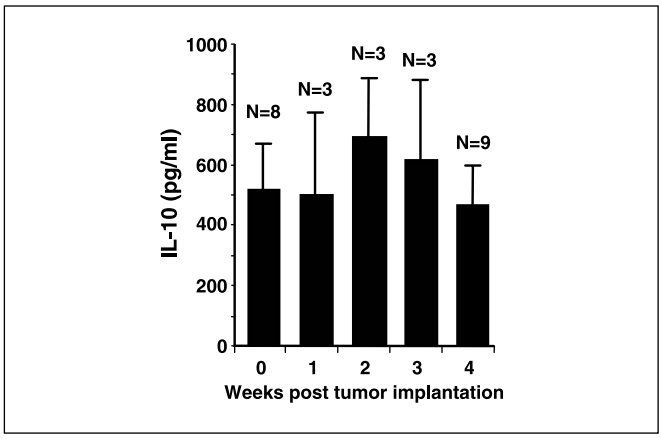

Figure 5. Macrophages from tumor bearers do not produce significantly higher amounts of IL-10 as compared with those from normal mice. IL-10 protein concentration was measured by ELISA in 24-hour supernatants of macrophages from normal mice and animals bearing 1-, 2-, 3-, and 4-week tumors cultured with and without LPS (10 μg/mL). Results for different number of animals (*n*) in each group; *columns,* mean of three replicates; *bars,* SD.

turnover of p65 and c-rel and their removal from proinflammatory gene promoters. Yet, our results showed no significant differences between the levels of IKKα expression in either cell type. Therefore, it is unlikely that deficiencies in the phosphorylation of IκBα account for the decreased binding activity of NFκB observed in T-PEM.

If a down-regulated nuclear translocation of NFκB and C/EBP occurs, this could be another underlying cause of the deficient binding activities of these transcription factors in T-PEM. Our data show that there is a severely reduced nuclear translocation of NFκB p65, p50, and c-rel in T-PEM. Furthermore, a diminished nuclear translocation of C/EBPα and β was also observed in T-PEM.

Our studies provide evidence that the mammary tumor used in our studies induces a down-regulated expression of transcription factors that may affect macrophage functions in T-PEM, favoring tumor escape. Sica et al. (28) have shown in different experimental and human tumors that increased IL-10 production, via the inhibition of NFκB activity, is the cause of decreased levels of proinflammatory IL-12 observed in tumor-associated macrophages. Whether the deficient NFκB binding activity observed by these authors is due to decreased expression and nuclear translocation of NFκB in these cells is not known. IL-10 is an inducer of a distinct activation program in macrophages (M2 macrophages) that down-regulates inflammatory responses and adaptive Th1 immunity and promotes angiogenesis and tissue remodeling (26). As shown in Fig. 5, we have not observed such an increase in IL-10 production in peritoneal macrophages from mice bearing D1-DMBA-3 tumors with different degrees of progression, suggesting that they are not of an M2 phenotype. The finding of these down-regulations in T-PEM, which in contrast to tumor-associated macrophages are not in direct contact with the tumor, further underscores the potency of tumor-derived factors that are capable of acting far from the tumor milieu on peripheral macrophages.

In contrast to cancer cells, which usually have an up-regulated NFκB activity (34), our studies show a decreased activity and diminished NFκB and C/EBP protein expressions in macrophages from tumor hosts. We propose that tumor-derived factors may operate as modulators of NFκB and C/EBP expression, two transcription factors that are crucial for an adequate immune response, providing a novel mechanism of tumor evasion.

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