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Down-Regulated Expression of TWEAK mRNA in Acute and Chronic Inflammatory Pathologies

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TWEAK is a newly identified member of the Tumor Necrosis Factor (TNF) family of proteins which are involved in many immunoinflammatory mechanisms. The putative role of TWEAK in inflammation was analyzed in mice treated with lipopolysaccharide (LPS), a strong inducer of the immuno-inflammatory responses. TWEAK mRNA rapidly disappeared in all the tissues tested. Analysis of LPS-treated thioglycolate-elicited peritoneal macrophages revealed that the rapid loss of TWEAK mRNA was due to its active destabilization. In chronic pathologies like autoimmune hemolytic anemia in the NZB mouse strain or systemic lupus erythematosus (SLE) in the BXSB mouse strain, TWEAK mRNA was shown to be reduced concomitantly to the development of chronic autoimmune diseases. These results demonstrated that TWEAK mRNA, contrary to TNF mRNA, is stable, ubiquitously distributed in tissues, and is down-regulated after LPS treatment or in chronic inflammation, suggesting that TWEAK could be an important factor, along with TNF, in acute and chronic inflammations.

Key Words: **TWEAK; TNF; SLE; autoimmune hemolytic anemia; BXSB; NZB.**

The proteins of the tumor necrosis factor (TNF) family are involved in the development and the regulation of the immune system and multiple inflammatory responses (1). We have recently identified TWEAK, as a new member of the TNF family (2), which is a secreted protein, like tumor necrosis factor α (TNFα) and lymphotoxin α (LTα). It promotes the death of a restricted number of tumor cell lines through Apo3 receptor or through the induction of TNF and TNF receptor (2–4), and stimulates the proliferation of endothelial cells (5) and the secretion of IL-8 and IL-6 in HT-29 cells and in astrocytes (2, 6). Contrary to TNF α and LT α mRNAs which are inducible in restricted organs, TWEAK mRNA is present in most human and mouse tissues. This suggested that TWEAK could have a specific role in inflammatory diseases. In this paper we analyzed the regulation of TWEAK mRNA in acute and chronic inflammation.

Abbreviations used: TWEAK, TNF-like weak inducer of apoptosis; TNF-α, tumor necrosis factor-α; LPS, lypopolysaccharide; LT-α, lymphotoxin-α; SLE, systemic lupus erytematosus.

MATERIALS AND METHODS

C57BL/6, NZB, BXSB and NZB.Yaa mice were bred in our facility. C57BL/6 mice were treated with 100 μg of LPS in 100 μl of PBS by retro-orbital injection.

Peritoneal macrophages from C57BL/6 mice were isolated as already described (7). They were cultured in DMEM containing 5% FCS for 24 h before the addition for the indicated time of either LPS (100 ng/ml) or Actinomycin D (5 μg/ml) or cycloheximide (10 μg/ml) or mTWEAK (100 ng/ml, a gift from J. Browning, Biogen Inc., Cambridge).

The cells or the tissues were lysed in TriZol (Life Technologies) and RNA were extracted as indicated by the manufacturer. Northern blots were hybridized successively with ^{32}P-labeled mTWEAK, mTNF and GAPDH riboprobes as already described (7). The 500-bp mTWEAK riboprobe was as described (2). Hematocrits were directly determined from blood samples as described previously (8).

Lupus-like glomerulonephritis (GN) was scored on a 0–4 scale based on the intensity and extent of histological changes, according to Pirani and Salinas-Madrigal (9).

RESULTS AND DISCUSSION

Down-Regulation of TWEAK mRNA Expression in Mouse Tissues and Peritoneal Macrophages Following Treatment with LPS

C57BL/6 mice were injected intravenously with 100 μg of LPS in PBS. At different times, the mice were killed and tissues were processed for RNA extraction. Northern blots were hybridized with ^{32}P-labeled mTWEAK antisense riboprobe. 3–5 h after LPS injection, TWEAK mRNA had virtually disappeared in kidney, heart, and lung (Fig. 1). Similar results were obtained with liver and spleen displaying a lower steady state level of TWEAK mRNA (not shown). This

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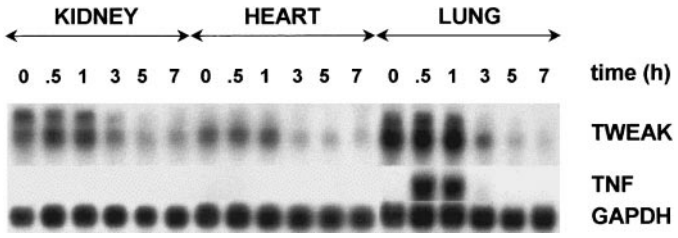

FIG. 1. Time course analysis of TWEAK mRNA expression in kidney, heart, and lung from mice injected with LPS. C57BL/6 mice were treated by retro-orbital injection with 100 μg of LPS. At the time indicated, mice were killed, organs were taken and RNAs were purified with TRIZOL (Life Technologies). Northern blot was hybridized successively with ^{32}P-labeled mTWEAK, mTNF and GAPDH riboprobe.

markedly contrasted to a rapid induction of TNF mRNA in lung from LPS-injected mice (Fig. 1).

To determine the half-life of TWEAK mRNA, 24 h-adherent peritoneal macrophages from mice treated with thioglycolate were incubated for the time indicated with either LPS, the transcription inhibitor actinomycin D or the protein synthesis inhibitor cycloheximide. Consistent with the results obtained with *in vivo* experiments, treatment with LPS rapidly decreased the level of TWEAK mRNA (Fig. 2). Densitometric evaluation indicated a half-life of 2.5 h. TWEAK mRNA from actinomycin-treated cultures had an estimated half-life of 7 h. Thus, LPS induces a specific reduction in steady-state level of TWEAK mRNA. Most cytokine mRNAs including TNF mRNA are known to accumulate in culture with a protein synthesis inhibitor. TWEAK mRNA was, however, stable or even slightly decreased in cycloheximide-treated cultures, indicating that in normal conditions, degradation of TWEAK mRNA is apparently independent of ongoing protein synthesis. Thus, the loss of TWEAK mRNA in mouse tissues and macrophages treated with LPS is probably linked to the activation of a destabilizing factor acting on the AU-rich element of TWEAK mRNA

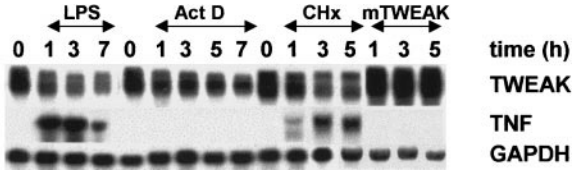

FIG. 2. Northern blot analysis of TWEAK mRNA in mouse peritoneal macrophages. Peritoneal macrophages were obtained from C57BL/6 mice pretreated with thioglycolate, and cultured for 1 h in 10 cm petri dishes at 10^6 cells/ml. Nonadherent cells were washed away and the adherent cells, mainly macrophages, were cultured for 24 h in DMEM containing 5% FCS. LPS (100 ng/ml), actinomycin D (5 μg/ml), cycloheximide (10 μg/ml), or mTWEAK (100 ng/ml) were added. At the indicated times, the cells were lyzed in TRIZOL and RNAs were purified. Northern blots were hybridized with ^{32}P-labeled mTWEAK, mTNF or GAPDH riboprobes.

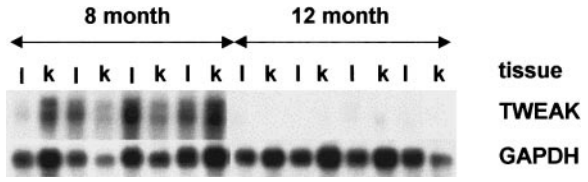

FIG. 3. Northern blot analysis of TWEAK mRNA in NZB mice. Male NZB mice at 8 months of age or at ~12 month of age were killed, liver and kidney were taken and processed for RNA purification. Northern blot was hybridized with ^{32}P-labeled mTWEAK or GAPDH riboprobes.

(2) or to a higher turnover of TWEAK mRNA because of a rapid increase of its translation. In contrast, we observed that murine rTWEAK at 100 ng/ml rapidly increased (2- to 5-fold) the steady state-level of TWEAK mRNA without any effect on TNF mRNA (Fig. 2).

Down-Regulation of TWEAK mRNA Expression in Mice Developing Chronic Autoimmune Diseases

The TWEAK gene belongs to human chromosome 17p13. This corresponds to chromosome 11.40 in the mouse (2). This region apparently possesses candidate genes (*Lbw8* and D11Mit84/*Nba*) involved in murine lupus-like autoimmune disease (10, 11). We thus searched for a possible involvement of TWEAK in autoimmunity by investigating the relationship between TWEAK mRNA expression and autoimmune pathology. We focused on two models, NZB and BXSB strains. NZB mice naturally develop autoantibodies, mainly against red blood cells and the mortality arises from a severe hemolytic anemia (11). BXSB mice possess in the Y chromosome a mutant gene, *Yaa* (Y chromosome-linked autoimmune acceleration), capable of accelerating a lupus-like autoimmune disease (12).

The expression level of the TWEAK mRNA in NZB mice was assessed in relation to the development of autoimmune hemolytic anemia. As shown in Fig. 3, TWEAK mRNA were undetectable in liver and kidney from ~12-month-old anemic NZB mice (with hematocrit ranging from 24 to 30%), compared to 8-month-old mice having a normal range of hematocrit values (41–45%).

In lupus-prone BXSB male mice bearing the *Yaa* mutation, a significant reduction in the level of TWEAK mRNA was also seen in kidney from 9-month-old males developing severe lupus-like glomerulonephritis (Fig. 4). Notably, such a reduced level of TWEAK mRNA abundance was not observed in 9-month-old female BXSB mice who fail to develop significant autoimmune syndrome during the first year of life (13). Since the *Yaa* mutation markedly accelerates the progression of autoimmune hemolytic anemia and lupus-like glomerulonephritis in NZB mice (14), we also determined the expression level of TWEAK mRNA in kidney from NZB male mice bearing the *Yaa*

mutation (NZB.*Yaa*). Although their glomerular lesions were still limited, no or few TWEAK mRNA was detectable in 3-month-old NZB.*Yaa* males, which contrasted to the abundance of TWEAK mRNA in 3- to 6-month-old female counterparts (Fig. 5).

The *Yaa* gene apparently accelerates the loss of TWEAK mRNA. We noted that as compared with NZB.*Yaa* male mice, only a partial decrease was observed in male BXSB *Yaa* mice developing severe renal disease. This in part correlates with the severity of lupus-like autoimmune syndrome developing in these two strains of mice, in which the mean mortality arises at 8 months of age for BXSB males but at 5.5 months of age for NZB.*Yaa* males (13, 14). However, it should be stressed that the loss of TWEAK mRNA preceded the onset of severe autoimmune syndrome in NZB.*Yaa* mice, indicating that the disappearance of TWEAK mRNA is not the consequence of severe pathological manifestations. The marked difference in the extent of down-regulation of TWEAK mRNA between NZB and BXSB mice is likely to be related to differences in the genetic background of these two strains of mice.

The results described in this paper clearly shows an inverse correlation between TWEAK mRNA level and inflammation. Since TWEAK exhibits proinflammatory activities (IL-8, IL-6, chemokine inductions) (2, 6, and Chicheportiche *et al.*, manuscript in preparation), one can speculate that a rapid production of TWEAK during inflammation may be assured by the unleashing of the translation of TWEAK mRNA abundantly present throughout the body tissues without induction of its *de novo* transcription. Consequently, the turnover of TWEAK mRNA may be markedly accelerated following an inflammatory stimulation. The TWEAK mRNA abundance could be, however, restored and maintained through the mechanism of TWEAK autocrine stimulation. In addition, we have recently shown that full length TWEAK transfected 293-EBNA cells express little TWEAK, unlike other members of the TNF family (2). Such a low translational capacity under physiological conditions could also explain the presence of relative abundance of the basal level of TWEAK mRNA in tissues, thus maintaining a homeostatic regulation.

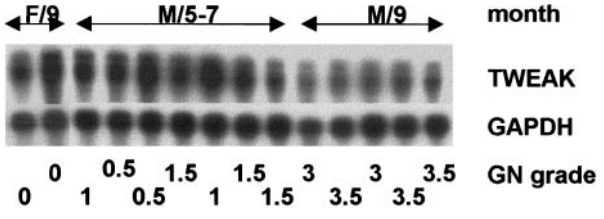

FIG. 4. Northern blot analysis of TWEAK mRNA in BXSB mice. Kidneys from BXSB male and females of different ages were processed for RNA extraction. Northern blot was hybridized with ^{32}P-labeled mTWEAK or GAPDH riboprobes. Glomerulonephritis (GN) was scored as described under Materials and Methods.

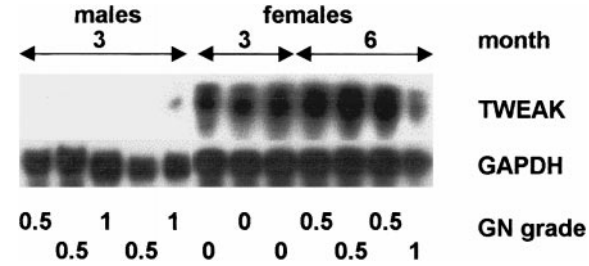

FIG. 5. Northern blot analysis of TWEAK mRNA in NZB.*Yaa* mice. Kidneys from male (*Yaa*) and female NZB mice at 3 and 6 months of age were processed for RNA extraction. Northern blot was hybridized with ^{32}P-labeled mTWEAK or GAPDH riboprobes. Note that although glomerular lesions in 3-month-old NZB.*Yaa* male mice were still limited, 50% of them died of severe lupus-like GN around 5–6 months of age.

In view of a proinflammatory activity of TWEAK and remarkable modulation of its mRNA expression during acute and chronic inflammations, screening of TWEAK in sera from different pathological conditions and in cultured supernatants from different types of cells should undoubtedly give invaluable information on the role of TWEAK in inflammatory and autoimmune pathologies.

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