Tristetraprolin Limits Inflammatory Cytokine Production in Tumor-Associated Macrophages in an mRNA Decay–Independent Manner

Franz Kratochvill\textsuperscript{1,2}, Nina Gratz\textsuperscript{1}, Joseph E. Qualls\textsuperscript{1,2}, Lee-Ann Van De Velde\textsuperscript{1,2}, Hongbo Chi\textsuperscript{3}, Pavel Kovarik\textsuperscript{3}, and Peter J. Murray\textsuperscript{1,2}

Abstract

Tristetraprolin (TTP) is an inducible zinc finger AU-rich RNA-binding protein essential for enforcing degradation of mRNAs encoding inflammatory chemokines and cytokines. Most studies on TTP center on the connection between mRNA half-life and inflammatory output, because loss of TTP amplifies inflammation by increasing the stability of AU-rich mRNAs. Here, we focused on how TTP controls cytokine and chemokine production in the nonresolving inflammation of cancer using tissue-specific approaches. In contrast with model in vitro macrophage systems, we found constitutive TTP expression in late-stage tumor-associated macrophages (TAM). However, TTP's effects on AU-rich mRNA stability were negligible and limited by constitutive p38\textsuperscript{\alpha} MAPK activity, which was the main driver of proinflammatory cytokine production in TAMs at the postranscriptional level. Instead, elimination of TTP caused excessive protein production of inflammatory mediators, suggesting TTP-dependent translational suppression of AU-rich mRNAs. Manipulation of the p38\textsuperscript{\alpha}–TTP axis in macrophages has significant effects on the growth of tumors and therefore represents a means to manipulate inflammation in the tumor microenvironment. Cancer Res; 75(15): 3054–64. ©2015 AACR.

Introduction

Nonresolving inflammation (NRI) underpins most chronic diseases, including obesity, autoinflammatory diseases, atherosclerosis, chronic responses to implanted medical devices, chronic or latent infections, and cancer (1). NRI is caused by aberrant molecular pathways underpinning NRI and how to mitigate their effects in chronic diseases.

In resolving inflammation triggered by TLR signaling, the TNF mRNA is rapidly produced from poised Pol II at the Tnf promoter (3–5). TNF protein is made and secreted, followed by a downregulation phase where the tristetraprolin (TTP) zinc finger protein, also induced in the early phase of TLR signaling, plays a decisive role in eliminating TNF mRNA (6). In model, in vitro systems such as BMDMs the TNF–TTP pathway is controlled by two key factors, p38\textsuperscript{\alpha} MAPK and II10 (7–9). p38\textsuperscript{\alpha} activated by TLR signaling phosphorylates TTP thereby blocking its function and sustaining TNF output (9, 10). In the resolution phase, DUSP proteins dephosphorylate p38\textsuperscript{\alpha}, which leads to dephosphorylation of TTP and a rise in TTP activity to eliminate TNF mRNA. II10, stimulated by TLR signaling to act in an autocrine way, further enhances removal of the TNF mRNA because it drives TTP and DUSP1 expression in a STAT3-dependent way (7, 11, 12).

To begin to understand the signaling pathways involved in regulating chronic inflammation in tumor-associated macrophages (TAM), we used transplantable tumor models with predictable temporal inflammatory responses that can be tracked by flow cytometry and that are amenable to the use of genetics to probe signaling pathways and how they become dysregulated.

We focused on how TTP regulates cytokine and chemokine production because inflammatory cytokines are the key drivers of chronic inflammation such as cancer. A key tactic was using a conditional knockout allele of Zfp36 (encoding TTP) to avoid complications of systemic inflammation present in conventional Zfp36\textsuperscript{-/-} mice early in development (13–15). Our results show that TAM maintains macrophages on a “knife-edge” between counterregulation of inflammation and death. Our results also suggest TTP primarily regulates steps beyond mRNA stability such as mRNA translation in macrophages associated with cancer inflammation.

Materials and Methods

Mice

C57BL/6, Tie2-Cre (B6.Cg-Tg(Tie2-cre)1Ywa\textsuperscript{\textsuperscript{y}}), and LysM-Cre (Lyz2\textsuperscript{am}Cre\textsuperscript{Ifo/J}) mice were obtained from The Jackson Laboratory. TTP\textsuperscript{KO} (Zfp36\textsuperscript{-/-}) mice were a gift of P. Blackshear (National Institute of Environmental Health Sciences, Research Triangle Park, NC). The TTP\textsuperscript{MK} and Mapk14\textsuperscript{lox/lox} mice have been described elsewhere (15, 16). Th-MYCN transgenic (tg; ref. 17) and Rb\textsuperscript{lox/lox}, p53\textsuperscript{lox/lox}, Osx-Cre\textsuperscript{+} (mixed background) mice (18) were obtained from M. Dyer, St. Jude Children’s Research Hospital,
Memphis, TN. For experiments, sex-matched mice were grouped and used between 6 and 10 weeks. All mice were on a C57BL/6 background unless indicated otherwise. Mice were used within the Animal Resource Center according to protocols approved by the Institutional Animal Care and Use Committee of St. Jude Children's Research Hospital.

Zymosan A induced peritonitis

Chronic peritonitis was induced by i.p. injection of 10 mg/mouse of zymosan A (Sigma) as described (19) and CD11b+ cells were purified by Miltenyi magnetic beads according to the manufacturer's protocol.

TAM collection from solid tumors

Solid tumors were minced and digested with 5 mL of fresh Tumor Digestion Media as described in the Supplementary Materials and Methods. The suspension was then passed through a 70-μm strainer and cells were overlaid on a 35%/60% percoll gradient. After centrifugation cells were collected from the 35%/60% interphase and TAMs were isolated by CD11b purification.

Figure 1.

NRI-associated macrophages share a common mode of development and express proinflammatory cytokines. A and B, flow-cytometry plots of CD11b+Ly6G− myeloid cells isolated from EG7 thymomas (A) or zymosan induced peritonitis (B) at different time points as indicated. Plots are representative for at least two experiments. Gates A to D indicate TAM or peritonitis-associated macrophage (PAM) populations used for sorting experiments or to measure cytokine production by intracellular flow cytometry. C, quantitative analysis of EG7 TAM populations A and C (gated as in A) over time. Values are from one of two independent experiments (n ≥ 4); error bars, SEM. D, quantitative analysis of cytokine production by flow cytometry of EG7 TAM populations A to C gated as shown in A. Values from individual mice are either depicted as the percentage of positive cells (left) or median fluorescence intensity (MFI, middle) of TNF or IL1α-positive (TNF+ and IL1α+) compared with negative (TNF− and IL1α−) macrophages within population A to C. Example plots are shown (right) indicating the gating strategy for cells considered positive and negative. Unstained control, gray. Data are mean ± SEM of two independent experiments (n ≥ 2).
using Miltenyi magnetic beads according to the manufacturer’s protocol.

**Tumor models—in vivo**

EG7 lymphoma line (ATCC) originating from the thymus (EL4), stably expressing OVA protein was tested for pathogen contamination before injection. A total of \(3 \times 10^6\) EG7 cells were injected s.c. into the flank of recipient mice and harvested 12 days later unless indicated otherwise. Orthotopically grown gliomas (20) and thymomas were harvested from mice between 1.5 and 2 weeks posttransplant. Spontaneous neuroblastomas from Th-MYCN transgenic (tg) and osteosarcomas collected from \(Rb^{fl/ox}/p53^{fl/ox}/Osx-Cre\) mice (18) were detected by ultrasound or visual inspection of tumors on the long bones or jaws, respectively.

**Antibodies and immunoblotting**

For flow cytometry: Anti-mouse MHC II (I-A/I-E; clone M5/114.15.2, cat. no. 12-5321-83), anti-mouse Ccl3 (clone DNT3CC, cat. no. 12-7532-80), anti-mouse II10 (clone Jess-16E3, cat. no. 11-7101-81), anti-mouse II1 (clone ALF 161, cat. no. 12-7011-81), anti-mouse IL1β (clone NJTEN3, cat. no. 12-7117-80) all eBioscience, anti-mouse TNFα (cat. no. 554419; PharMingen), p-p38 MAPK (T180/Y182; clone D3F9, cat. no. 4511S) and Santa Cruz Biotechnology (clone A-12, cat. no. sc-7972), respectively. Whole-cell extracts were prepared and assayed by Western blotting as described (7) using 4% to 15% Tris-HCl gels (Criterion; Bio-Rad). Equal loading was controlled by anti-mouse GRB2 antibody (cat. no. 610112; BD Biosciences).

**Enzyme-linked immunosorbent assay**

Murine TNF production was measured from supernatants of TAMs resting on tissue-culture plates for 6 hours using preoptimized Capture and Detection antibodies (both eBioscience).

**Results**

TAMs produce proinflammatory cytokines during all stages of development

Blood-derived monocytes invade almost all types of solid tumors where they develop into macrophages. Macrophages are
the most numerous myeloid cells in chronic inflammation, and the source of many key proinflammatory cytokines and chemokines that drive inflammation forward (21). To dissect macrophage function in cancer inflammation, we developed methods to reliably distinguish macrophages at different points in their development from neutrophils and eosinophils. Building on a
system developed by Movahedi and colleagues, we identified three monocyte-derived populations of macrophages in solid tumors as well as in chronic zymosan peritonitis, a complementary model for chronic inflammation where monocytes infiltrate the inflammatory site (Fig. 1A and B; ref. 22). The "A" population comprises inflammatory monocytes (Ly6C^hi and MHCII^-0^) and the "B" population contains monocyte-derived inflammatory precursors (Ly6C^hi and MHCII^+/0^).
A hallmark of chronic inflammation is constitutive cytokine and chemokine production promoted by the presence of the inciting entity. A crucial question in understanding the relationships between cytokine and chemokine-producing cells in chronic inflammation is whether negative feedback loops counteracting disease-causing inflammation are inactive, and how they become dysregulated. We therefore asked whether TAMs expressed inflammatory cytokines and whether their expression pattern was linked to the "A, B, and C" development pathway. Although we found highest expression of TNF and IL12 in the "B" fraction, all macrophage subsets expressed readily detectable amounts of both cytokines (Fig. 1D). For example, 36% of the "B" fraction were TNF⁺ (Fig. 1D).

AU-rich element-binding proteins are highly expressed in TAMs

TTP is essential for mediating cytokine and chemokine mRNA decay in inflammation. Indeed, global absence of TTP...
Figure 6.
p38α regulates TTP expression and activity in TAMs. A, EG7 TAM RNA from WT or p38αΔH tumor-bearing mice was analyzed by qRT-PCR. Expression values from individual mice combined from three experiments (n ≥ 13) were normalized to GAPDH and the mean WT within each experiment is depicted as mean indicated by black lines; error bars, SEM. B and G, mRNA stability measured by qRT-PCR as in Fig. 4D in EG7 TAMs from WT (Mapk14fl/fl) and p38αΔH animals (B) and in unstimulated (−) or SB203580 (SB)-treated WT EG7 TAMs (G). SB treatment was started concomitant to actinomycin D-imposed transcriptional blockade. Values from individual mice were normalized to GAPDH from either three to five (n ≥ 10, B) or one of two (n ≥ 7; G) experiments, respectively. Black lines, mean per group; error bars, SEM. C, cytokine expression analyzed by flow cytometry of EG7 TAMs (gated on CD11b+). Values from individual mice are the percentage of CD11b+ cells (n = 6) representing one of two experiments with the mean depicted as a black line; error bars, SEM. D, cytokine production evaluated by intracellular flow cytometry in lethally irradiated mice reconstituted with a mixture of WT (CD45.1) and p38αΔH (CD45.2) bone marrow (n = 4). Depicted values represent percentage of positive cells of CD11b+ TAMs gated for CD45.1 or CD45.2 as shown (left); error bars, SEM. E, EG7 tumor mass 12 days posttransplantation of EG7 cells into WT or p38αΔH animals. (Continued on the following page.)
in all cells causes systemic inflammatory pathology because multiple inflammatory cytokines, including TNF and IL23, are produced in excess (13, 14). TTP's target mRNAs form a hierarchy dependent in part on the number and type of AUU elements in the mRNA 3'-untranslated region (25). As both TNF and IL1α mRNAs are TTP targets (6, 15, 26), but highly expressed in tumor inflammation-associated macrophages, we suspected sustained cytokine production by TAMs was caused by failure of TTP expression. Paradoxically, we found TTP mRNA and protein was highly expressed in TAMs, and expression increased as cells developed to the “C” population (Fig. 2A and B). Although TTP was expressed, cytokine production was high (Fig. 1D). High expression of TTP was not an artifact of our experimental system because high amounts of the Zfp36 mRNA (encoding TTP) and TTP protein were detectable in macrophages from different cancer types, and were expressed at higher amounts than LPS-stimulated BMDMs (Fig. 2C). Interestingly, mRNAs encoding two TTP-related zinc finger mRNA-destabilizing proteins, Zfp36L1 and Zfp36L2, were also highly expressed in tumor macrophages, being among the top 2% genes (Fig. 2D; F. Kratovich and colleagues, manuscript submitted; GEO accession number GSE59047). Given TTP, Zfp36L1, and Zfp36L2 were highly expressed in TAMs, but cytokine expression was correspondingly high, we concluded that mRNA degradation processes are ineffective at eliminating inflammatory cytokine mRNAs in tumor macrophages.

TTP limits the production of inflammatory cytokines in TAMs

We next asked whether TTP was required for regulating cytokine and chemokine production. To do this, we generated TAMs from mice where TTP is specifically and efficiently eliminated from macrophages and neutrophils using the LysM-Cre deleter (called TTPΔlox mice; Fig. 3A). This tactic enabled us to avoid the effects of systemic inflammation in the Zfp36fl/fl mice where the absence of TTP increases numerous proinflammatory cytokines and results in early death (13, 14). We examined tumors in the TTPΔlox mice and found they were smaller and contained more dying cells as compared with tumors from control mice (Fig. 3B–D). TAMs from TTPΔlox mice had increased numbers of macrophages positive for TNF, Ccl3, Il1α, and Il1β (Fig. 3E and F). In addition, TAMs isolated from TTPΔlox animals secreted approximately 2.5-fold more TNF when rested on tissue culture dishes for 6 hours than WT TAMs (Fig. 3G). We further compared the TNF production in TAMs with resting or LPS stimulated BMDMs. TAMs produced more TNF than resting BMDMs and approximately one fourth of TAMs with resting or LPS stimulated BMDMs. TAMs produced more TNF when rested on tissue culture dishes for 6 hours than WT TAMs (Fig. 3G). We further compared the TNF production in TAMs with resting or LPS stimulated BMDMs. TAMs produced more TNF than resting BMDMs and approximately one fourth of TNF secreted by an equivalent number of homogeneous BMDMs (Fig. 3G). Production of the anti-inflammatory cytokine and TTP target Il10 (27) was unaffected in terms of number of Il10 producing macrophages or intensity of staining (Fig. 3E and F). Collectively, these data suggest TTP is required to temper the already high cytokine and chemokine production in the inflammatory milieu of chronic inflammation.

When TTP is genetically eliminated in macrophages, cytokine, and chemokine output further increases relative to the already high amounts in controls, which is linked to increased cell death and decreased tumor size.

TTP-dependent mRNA decay is limited in TAMs

TTP increases the instability of AU-rich mRNAs (6). Given high expression of TTP in macrophages associated with chronic inflammation, we asked whether TTP regulated AU-rich mRNA stability. We measured steady-state mRNA amounts of AU-rich inflammatory mRNAs in the total CD11b+ myeloid fraction from tumors and found no differences between controls and TTPΔlox cells (Fig. 4A). TTP has also been implicated in regulating the transcription status of certain genes, possibly compensating for alterations at the mRNA stability level (28). We therefore assessed transcription by detecting the unspliced mRNA primary transcripts of inflammatory mRNAs in WT and TTPΔlox TAMs. Similar to total mRNA amounts we detected no significant changes between control and TTPΔlox cells (Fig. 4B). Furthermore, focusing on the TAM subsets where TTP expression is highest, we also failed to detect significant effects on total amounts of normally unstable TTP targets with the exception of the TNF mRNA, which is known to be affected by TTP-dependent mRNA decay even under conditions where TTP function is limited (Fig. 4C; ref. 15). When we estimated the decay rates of TTP targets in control or TTPΔlox TAMs, we found significant effects on stability only for TNF and KC, the AU-rich mRNAs that show the highest effects of TTP activity in model systems (Fig. 4D; ref. 15). However, the data in Fig. 4A–C indicate effects of TTP on mRNA stability were marginal, with a total quantity of TNF and KC mRNAs were equivalent between control and TTPΔlox TAMs. Therefore, TTP controls chemokine and cytokine production at a level above transcription or mRNA stability. Thus, tumor macrophages had high TTP protein, and TTP was required to inhibit inflammatory cytokine and chemokine production, but not their cognate mRNA amounts or stability.

p38α is constitutively activated in NRI

If TTP is highly expressed in chronic inflammation, why is AU-rich mRNA degradation inefficient? To begin to answer this question, we noted TTP activity toward its AU-rich mRNA substrates is negatively regulated by phosphorylation on specific serine and threonine residues (9). Phosphorylation of TTP could, therefore, account for why AU-rich mRNA quantity and stability were unaffected in macrophages. To test this, we assessed pTTP in TAMs and observed a characteristic “smear” of TTP protein similar to that found in LPS-stimulated BMDMs (Fig. 5A; ref. 8). In the latter, pTTP increases to stabilize inflammatory cytokine and chemokine mRNAs, enhancing their production, after which active (unphosphorylated) TTP rises to eliminate AU-rich mRNAs to return macrophages to a “ground” state (7, 10, 15). In accordance with high amounts of pTTP associated with inactive TTP-dependent mRNA decay, the stability of the TNF mRNA was increased in TAMs isolated from different tumor models.

(Continued) Values from individual mice combined from three experiments are shown, with the black line representing the mean; error bars, SEM. F, qRT-PCR analysis of TTP and TTP family members in E7 TAMs from WT and p38α−/− mice. Expression values from individual mice (n = 14) were normalized to GAPDH and the corresponding mean WT from three independent experiments. Mean, black line; error bars, SEM. H, mRNA decay measured in WT and TTPΔlox E7 TAMs with and without SB treatment as in G. Values of individual mice are depicted as the percentage of mRNA stability after SB treatment relative to the corresponding stability in untreated WT or TTPΔlox TAMs. Values were normalized to GAPDH and the corresponding untreated TAM sample (n ≥ 9) and were combined from two experiments. Black line, mean; error bars, SEM.

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compared with resting BMDCs where TTP activity is known to be high (Fig. 5B, ref. 15).

TTP inhibition was reported to be mediated by p38α MAPK, the predominant MAPK in macrophages, and to be blocked by phosphaestases, which act on p38α, and thus indirectly promote TTP function (26, 29). We therefore investigated how the p38α–TTP cycle works in chronic inflammation. First, we observed overall high p-p38α in tumor-derived macrophages despite varying degrees of total p38α (Fig. 5C). p38α activation was constitutive and not a transient effect from the isolation procedure as we could still detect strong p38α phosphorylation in TAMs rested on tissue culture dishes for 3 hours (Fig. 5D). Second, when we genetically eliminated p38α in macrophages with the LysM-Cre deleter(p38α<sup>fl/fl</sup>) as previously reported (30), we observed negligible depletion of p38α amounts, arguing conclusions drawn using this deleter system are difficult to interpret (Fig. 5E and F). Therefore, for subsequent experiments, we adjusted our tactics and used the pan-hematopoietic deleter Tie2-Cre (p38α<sup>M1</sup>), which produced a complete elimination of p38α (Fig. 5G and H).

**p38α drives chronic inflammation posttranscriptionally**

When we measured inflammatory cytokine and chemokine mRNA amounts in p38α-deficient TAMs, we observed no differences between controls and knockouts for transcripts such as TNF, IL1α, and IL6 and only minor differences for other selected transcripts (Fig. 6A). In the same system, mRNA stability in p38α-deficient TAMs was reduced for TNF and IL1β mRNA only (Fig. 6B). However, despite unaltered mRNA amounts p38α-deficient cells had a substantial reduction in the amounts of certain inflammatory cytokines such as TNF and IL1α, suggesting p38α is required for translation of inflammatory mediators (Fig. 6C, Supplementary Fig. S1). To exclude the possibility that the detected alterations are due to p38α deletion in nonmyeloid cells caused by the use of Tie2-Cre, we performed mixed radiation chimera experiments with 1:1 mixtures of control and cells caused by the use of Tie2-Cre, we performed mixed radiation chimera experiments with 1:1 mixtures of control and cells caused by the use of Tie2-Cre, we performed mixed radiation chimera experiments with 1:1 mixtures of control and. We therefore, for subsequent experiments, we adjusted our tactics and used the pan-hematopoietic deleter Tie2-Cre (p38α<sup>M1</sup>), which produced a complete elimination of p38α (Fig. 5G and H).

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As mentioned above, the mRNA stability of several TTP target transcripts was unaffected by p38α deletion (Fig. 6A and B). However, in accordance with in vitro studies (8), expression of TTP as well as the TTP-related proteins ZFP36L1 and ZFP36L2 was p38α dependent (Fig. 6F). Given a straightforward genetic means to address the role of p38α in regulating TTP activity was negated by the p38α dependence of TTP expression, we used a pharmacologic approach to block p38α. When we blocked p38α activity using the kinase inhibitor SB203580, we found AU-rich mRNAs were rapidly degraded (Fig. 6C). In TTP-deficient cells SB203580 treatment only partially increased mRNA decay. For instance, in WT TAMs, SB203580 treatment reduced TNF mRNA stability to approximately 43% of untreated TAMs compared with 64% in TTP<sup>M1</sup> TAMS, suggesting that p38α regulates mRNA stability in a TTP-dependent and -independent manner (Fig. 6H). Two conclusions can be drawn from these experiments. First, TTP-dependent mRNA decay in TAMs is blocked by p38α, consistent with previous data generated from in vitro systems. However, TTP is still able to limit protein production of target mRNAs, thereby counteracting inflammation. Thus, in tumor inflammation, TTP’s mRNA-degrading function is negated by p38α, but TTP is still required to temper inflammatory mediator output. Second, p38α is a driver of chronic inflammation at the posttranscriptional level. Our data suggest that p38α is constitutively activated in TAMs and resistant to the known negative feedback mechanisms described for acute inflammatory responses (12, 31, 32).

**Discussion**

Genetic experiments have established the basic parameters of TNF production in scenarios where the inflammatory signal is limited. First, p38α cooperates within the TLR signaling pathway to initiate Tnf expression and TNF production (33). In the subsequent resolving phase, a step-wise process cooperates to extinguish TNF production once TLR signaling tapers: DUSP proteins dephosphorylate p38α; the decreased p38α activity releases TTP inhibition (induced by TLR signaling) to degrade the TNF mRNA and IL10 (induced by TLR signaling) amplifies TTP expression because Zfp36 is a STAT3-dependent target of IL10 signaling (7, 12, 31, 32). Using conditional knockout approaches to minimize the effects of systemic inflammation, we found that in TAMs none of the step-by-step processes in TNF regulation occurs. Instead, the tumor microenvironment enforces constitutive p38α activation, leading to blockade of TTP activity and abatement of TNF mRNA removal.

TTP is generally considered to primarily control AU-rich mRNA stability (34). Although TTP also contributes to blocking translation of some mRNAs (35), our experiments suggest these two functions are differentially regulated within the tumor microenvironment. We found mRNA stability and total amounts of TTP target mRNAs were marginally affected in the absence of TTP. These results contrast with in vitro studies where TTP influences the stability and amounts of hundreds of AU-rich mRNAs (15, 27). However, the absence of TTP caused inflammatory protein amounts to increase, leading to increased death in the tumor microenvironment and decreased tumor volume. Therefore, in TAMs, we suggest that TTP mainly regulates steps beyond mRNA decay such as translation. A further implication of our studies is the two functions of TTP can be separated by the inhibitory actions of chronic p38α activity: TTP expression is constitutively high in TAMs and its effects on mRNA stability are blocked by p38α phosphorylation. However, in contrast with in vitro studies of acute inflammation where p38α blocks both TTP-dependent mRNA decay and translation (35), in TAMs TTP can retain its inhibitory activity on cytokine production. Our results, therefore, increase the understanding of gene and protein regulation in complex environments.

Our data indicate that p38α is constitutively active during NRI and drives the production of inflammatory cytokines at the posttranscriptional level. What signals lead to p38α activation in TAMs? Multiple NRI models have shown endogenous "alarmins" drive TLR and other inflammatory signaling. Alarmins include S100a8/a9, IL1α and HMGB1. Release of any of these can provoke TLR/IL1R signaling, leading to Tnf expression (36). However, a consequence of ongoing TLR/IL1R activation is p38α phosphorylation and subsequent TTP deactivation, leading to further TNF production and potential for additive TNFR signaling to perpetuate the overall response.
Our data implicate p38α activity as the nexus of inflammatory signaling in TAMs, regardless of what occurs downstream in the pathway. As long as p38α is active, all the subsequent steps leading to signaling abatement cannot occur in a step-wise manner. When we targeted p38α activity with the kinase inhibitor SB203580, inflammatory mRNAs were greatly reduced. Therefore, p38α is a central checkpoint in chronic inflammation. Given NRI inflammation occurs in virtually all chronic diseases, our results point to targeting two aspects of the disease: First, the inciting entity must be reduced, modified, or eliminated, coincident with targeting upstream inflammatory signaling at the level of p38α.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors’ Contributions

Conception and design: F. Kratochvill, P.J. Murray

Development of methodology: F. Kratochvill

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): J.E. Qualls, L.-A. Van De Velde

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): F. Kratochvill

Writing, review, and/or revision of the manuscript: F. Kratochvill, N. Gratz, J.E. Qualls, P.J. Murray

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): F. Kratochvill, L.-A. Van De Velde, H. Chi, P. Kovarik

Study supervision: P.J. Murray

Other (performed experiments): N. Gratz

Acknowledgments

The authors thank Perry J. Blackshear for generously providing the TTPlox and Michael A. Dyer for the Th-MYCN transgenic and B6R129S4-Cre (p53lox/lox, plox/Ox-Cre) mouse strains. The authors thank Martine F. Roussel for providing the glioma tumor model as well as Lidija Barbaric, Parker Ingle, Greg Lennom, and Richard Cross for technical assistance and Derek Gilroy and Roland Lang for discussion and advice.

Grant Support

F. Kratochvill is supported by the Austrian Science fund (J3309-B19). This work was supported by The Hartwell Foundation (P.J. Murray), Alex’s Lemonade Stand Foundation (P.J. Murray), NIH grants CA189990 (P.J. Murray), CA138064 (J.E. Qualls), NCI grant P30 CA1765, and the American Lebanese Syrian Associated Charities.

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Received January 22, 2015; revised April 21, 2015; accepted May 8, 2015; published OnlineFirst July 16, 2015.

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