Dysregulation of the inflammatory response can lead to diseases such as rheumatoid arthritis, Crohn’s disease, or septic shock. The cytokine IL-10 plays a role in limiting the magnitude, duration, and detrimental outcome of the inflammatory response (Moore et al., 2001). This was shown both with the generation of IL-10–deficient mice that spontaneously develop inflammatory bowel disease (Kühn et al., 1993) and with the identification of patients with homozygous mutations in the IL-10 receptor subunits who present with early-onset colitis (Glocker et al., 2009).

IL-10 plays a central nonredundant role in limiting inflammation in vivo. However, the mechanisms involved remain to be resolved. Using primary human macrophages, we found that IL-10 inhibits selected inflammatory genes, primarily at a level of transcription. At the TNF gene, this occurs not through an inhibition of RNA polymerase II (Pol II) recruitment and transcription initiation but through a mechanism targeting the stimulation of transcription elongation by cyclin–dependent kinase (CDK) 9. We demonstrated an unanticipated requirement for a region downstream of the TNF 3’ untranslated region (UTR) that contains the nuclear factor κB (NF-κB) binding motif (κB4) both for induction of transcription by lipopolysaccharide (LPS) and its inhibition by IL-10. IL-10 not only inhibits the recruitment of RelA to regions containing κB sites at the TNF gene but also to those found at other LPS–induced genes. We show that although IL-10 elicits a general block in RelA recruitment to its genomic targets, the gene–specific nature of IL-10’s actions are defined through the differential recruitment of CDK9 and the control of transcription elongation. At TNF, but not NFKBIA, the consequence of RelA recruitment inhibition is a loss of CDK9 recruitment, preventing the stimulation of transcription elongation.

© 2010 Smallie et al. This article is distributed under the terms of an Attribution–Noncommercial–Share Alike–No Mirror Sites license for the first six months after the publication date (see http://www.rupress.org/terms). After six months it is available under a Creative Commons License Attribution–Noncommercial–Share Alike 3.0 Unported license, as described at http://creativecommons.org/licenses/by-nc-sa/3.0/.

T. Smallie’s present address is Physiological Genomics and Medicine Group, Medical Research Council Clinical Sciences Center, Faculty of Medicine, Imperial College London, Hammersmith Hospital, London, W12 0NN, UK.

Correspondence
Lynn Williams:
lynn.williams@imperial.ac.uk

Abbreviations used: CDK, cyclin-dependent kinase; ChIP, chromatin immunoprecipitation; CTD, C-terminal domain; Pol II, RNA polymerase II; TSS, transcription start site; UTR, untranslated region.
studies of transcriptional effects have focused on the activity of NF-kB, a dominant transcription factor in the production of inflammatory cytokines (Foxwell et al., 1998; Udalova et al., 1998). However, it remains unclear whether IL-10 inhibits NF-kB activity (Wang et al., 1995; Schottelius et al., 1999; Denys et al., 2002; Murray, 2005; for reviews see Williams et al., 2004b; Grütt, 2005).

The variety of mechanisms proposed may reflect the use of mouse versus human systems or the use of genetically abnormal transformed cell lines, and we have therefore confined our study to the use of primary human macrophages. We have found that inhibition is not only exerted on the process of proinflammatory gene transcription but through a novel mechanism of elongation inhibition, controlled in a gene-specific manner through the actions of cyclin-dependent kinase (CDK) 9. IL-10 robustly inhibits RelA recruitment to kB sites at multiple proinflammatory genes, and this results in an inhibition only at genes that are rapidly induced at a level of elongation by CDK9. These findings shed new light on the gene specific nature of the IL-10 antiinflammatory response.

RESULTS AND DISCUSSION

We initially examined the effects of IL-10 on LPS-induced mRNA expression in primary human macrophages. IL-10 inhibited TNF and IkB-ζ mRNA production at early and late time points and IL-6, a secondary response gene, at 120 min (Fig. 1 A). IL-10 did not inhibit the production of IkB-α mRNA, which is consistent with the gene-specific effects previously described (Lang et al., 2002; Murray, 2005). A longstanding question has been whether IL-10 inhibits gene expression at the transcriptional (Murray, 2005) or posttranscriptional level (Schaljo et al., 2009), or perhaps both (Denys et al., 2002), in activated macrophages. Chromatin immunoprecipitation (ChIP) was used to measure accumulation of RNA polymerase II (Pol II) at distal regions of genes (Fig. 1 B), which is considered a hallmark of active transcription (Sandalval et al., 2004). LPS induced rapid accumulation of Pol II at a distal region of the TNF gene and slower accumulation at a distal region of the IL6 gene (Fig. 1 C), which is consistent with the identification of these as primary and secondary response genes (Hargreaves et al., 2009; Natoli, 2009; Ramirez-Carrozzi et al., 2009). IL-10 inhibited the accumulation of Pol II in the distal regions of TNF, IL6, and NFKBIA, but not NFKBIZ (Fig. 1 D), mirroring its effects on the corresponding mRNAs (Fig. 1 A). Nascent unspliced TNF transcripts were also quantified by PCR (Fig. S1 A) and were seen to accumulate with identical kinetics to those of mature TNF mRNA in these cells. As estimated by both of these methods, IL-10 decreased transcription of TNF (Fig. 1 D and Fig. S1 A). These findings in human macrophages are consistent with previous reports describing IL-10’s gene-specific effects on transcription (Lang et al., 2002; Murray, 2005).

Activation of transcription elongation has recently emerged as an important mechanism controlling the rapid induction of proinflammatory genes (Adelman et al., 2009; Hargreaves et al., 2009; Ramirez-Carrozzi et al., 2009). We therefore investigated whether IL-10 inhibits transcription elongation by comparing Pol II densities at the transcription start site (TSS) to the downstream region of TNF. As described by others (Adelman et al., 2009; Hargreaves et al., 2009; Ramirez-Carrozzi et al., 2009), Pol II was present at the TNF TSS under resting conditions and increased relatively weakly in response to LPS (Fig. 2 A). In contrast, it was scarcely detectable at the downstream region in resting cells and very strongly upregulated by LPS, which is consistent with control of gene expression largely through the release of a block to transcription elongation. IL-10 weakly inhibited the LPS-induced increase of Pol II at the TSS but strongly inhibited the increase of Pol II at the distal region (Fig. 2 A and Fig. S1 B). Phosphorylation of Ser2 of the Pol II C-terminal domain (CTD) by CDK9 occurs during the transition from the initiation of transcription to elongation (Price, 2000). We therefore performed ChIP using a phospho-Ser2–specific antibody. Where IL-10 did not inhibit the recruitment of total Pol II to the start of the TNF gene (Fig. 2 A; top left) it completely inhibited the phosphorylation of the Ser2 residue of the Pol II CTD (Fig. 2 A; bottom left). Therefore, at TNF the elongating form of Pol II is reduced by IL-10. When we conducted similar analysis at the NFKBIA gene, it was noted that neither the elongation of Pol II into the downstream region of the gene nor the phospho-Ser2 form of Pol II was decreased by IL-10 (Fig. 2 B).

We used the CDK9 inhibitor flavopiridol (Chao and Price, 2001) to confirm that the phosphorylation of Ser2 of the Pol II CTD controls elongation at TNF. Flavopiridol had negligible effects on total Pol II recruitment to the start of TNF in contrast to its inhibition of Pol II Ser2 phosphorylation (Fig. 2 C). Additionally, flavopiridol inhibited the accumulation of total Pol II at the downstream region of the TNF gene. These effects mirror IL-10’s inhibition of Pol II phospho-Ser2 and elongation into the downstream regions of the gene (Fig. 2 A).

Interestingly, transcription elongation of NFKBIA was also sensitive to flavopiridol (Fig. S1 D), indicating that although IL-10 had no effect on elongation of NFKBIA, inhibiting the kinase activity of CDK9 with flavopiridol elicited a broader effect. Therefore it is unlikely that IL-10 has inhibitory effects on the kinase activity of CDK9 (as was the case for flavopiridol). We therefore looked at the effect IL-10 had on the recruitment of CDK9 itself to the TNF and NFKBIA genes (Fig. 2 D). At the start of TNF, CDK9 was recruited in a stimulus-dependent manner and this was inhibited by IL-10 (mean 47.2%; SEM 6.5; n = 4). In contrast, at the start of NFKBIA, CDK9 was seen to be more constitutively associated, inducing only slightly in response to LPS and exhibiting no inhibition upon IL-10 stimulation. Recently, NFKBIA was described as a housekeeping primary response gene that is continuously transcribed in macrophages (Hargreaves et al., 2009), perhaps accounting for these differences. These differential effects on CDK9 recruitment between TNF and NFKBIA may underlie IL-10’s gene-specific, as opposed to general, inhibition of transcription elongation.

The current findings contradicted a previous observation from our laboratory, where the TNF 3′ untranslated region (UTR) was required for IL-10 to suppress the TNF reporter gene activity (Denys et al., 2002). This implicated a mechanism...
IL-10 inhibits proinflammatory gene transcription. [A] Cells were treated with LPS with or without IL-10 for 30 or 120 min. RNA was isolated and used in quantitative RT-PCR with probes specific for the indicated mRNA. [B] Position of PCR primers for detecting downstream Pol II at the TNF, IL6, NFKBIA, and NFKBIZ genes. [C] Cells were stimulated for the indicated times with LPS, followed by ChIP using Pol II antibodies (filled bars) or a rabbit isotype control (open bars) at downstream regions of the TNF and IL6 genes. [D] Cells were treated with LPS with or without IL-10 for 30 or 120 min and Pol II was measured by ChIP at the downstream regions of TNF, IL6, NFKBIZ, and NFKBIA. Data in A and C are expressed as mean ± SD of triplicate measurements for a single donor, representative of three independent experiments using different donors. Data in D are pooled data from three donors and are expressed as mean ± SEM. Statistical significance was assessed using the paired Student's t test. *, P < 0.05; NS (not significant).

of posttranscriptional control; however, on closer inspection, the construct in question, 5′luc3(1037) (Fig. 3 A), was found to include both the 785-bp TNF 3′UTR and an additional 250 bp of downstream genomic sequence (originally included in the construct for ease of cloning). We hypothesized that this 250-bp region, rather than the 3′ UTR, could have mediated the IL-10–dependent inhibition of 5′luc3(1037). Indeed, when this construct was compared with a 5′luc3(785) that lacked the additional genomic sequence, IL-10–dependent inhibition was lost (Fig. 3 A and Fig. S2 A). This also resulted in a partial loss of LPS responsiveness, indicating that the 3′ downstream region may contribute to TNF transcriptional control.

The downstream 3′ region of the TNF gene is known to contain the NF-κB binding site kB4 (Tsytosykov et al., 2007; Krausgruber et al., 2010). Although it is well documented that NF-κB plays a role in TNF transcription in macrophages
IL-10 inhibits transcription elongation at TNF. (A and B) Cells were treated with LPS with or without IL-10 for 30 min followed by ChIP using Pol II, phospho-Ser2-specific, or rabbit isotype control antibodies at the TSS or downstream regions of the TNF (A) or NFKBIA (B) genes. (C) Cells were left unstimulated or preincubated for 30 min with 500 nM flavopiridol, followed by LPS stimulation for 30 min, and then subjected to ChIP. (D) ChIP assay of CDK9 recruitment to the start of the TNF or NFKBIA genes upon stimulation with LPS with or without IL-10. Data are expressed as mean ± SD of triplicate measurements for a single donor, representative of three independent experiments using different donors.
IL-10 inhibits the recruitment of RelA to proinflammatory genes. (A–C) Adenovirus reporters based on the human TNF gene were infected into macrophages and stimulated with LPS with or without IL-10 for 4 h, after which luciferase assays were performed. Cells were either transfected with scrambled control oligonucleotides (siControl) or RelA-specific siRNA (siRelA; A), and cytoplasmic extracts were subjected to Western blotting using anti-RelA or tubulin antibodies to evaluate the extent of RelA knockdown (B) or cells were stimulated for the indicated times with LPS and total RNA was isolated and used as template in a quantitative RT-PCR gene expression assay for TNF mRNA (C). (D) Position of primers used in ChIP assays for the detection of RelA recruitment to the human TNF gene. NF-κB sites are indicated below the diagram, with the PCR targets represented by black lines with the length of the amplicons indicated above. (E) Cells were stimulated with LPS with or without IL-10 for 60 min, after which ChIP assays were performed using RelA-specific antibodies. Data are expressed as mean ± SD of triplicate measurements for a single donor representative of eight (A) or three (C and E) independent experiments using different donors.
IL-10 inhibits transcription elongation of TNF gene. [A and B] Cells were transfected with a siControl or siRelA oligonucleotides. Cells were stimulated with LPS with or without IL-10 for 1 h, after which ChIP was performed using RelA-specific antibodies and primers designed to the kB4 site of TNF(A) or Pol II-specific antibodies and primers specific for the TNF TSS or downstream regions (B). [C] Cells were treated with or without LPS for 1 h and ChIP was performed using Pol II-specific (left), phospho-Ser2 (middle), or CDK9 (right) antibodies and primers specific for the TNF TSS+1. Data are expressed as mean ± SD of triplicate measurements for a single donor representative of three independent experiments using different donors.

Considering that IL-10 inhibits RelA recruitment to the TNF gene, we wondered whether depletion of RelA in our experimental system could replicate the effects of IL-10 on transcription elongation. As expected, using an RNAi approach to deplete RelA, after LPS stimulation no RelA was detected to the TNF downstream region containing the kB4 site (Fig. 4 A). Concomitantly, RelA depletion had no significant effect on the recruitment of total Pol II to the start of TNF, yet it did result in an inability of LPS to induce its progression down the length of TNF (Fig. 4 B). This also coincided with a loss of inducible phosphorylation of Pol II at Ser2, and an inability to recruit CDK9 to the start of TNF in RelA-depleted macrophages (Fig. 4 C). These data show that artificial inhibition of RelA recruitment to its target kB sites replicates IL-10’s effects on TNF transcription elongation.

It is notable that IL-10 also inhibited the recruitment of RelA to the NFKBIA kB site, despite the fact that transcription of this gene is spared from inhibition. The fact that neither CDK9 recruitment nor Pol II Ser2 phosphorylation was inhibited by IL-10 at NFKBIA suggests that CDK9 recruitment here is independent of RelA. Certain aspects of these data bear resemblance to a study of the gene-specific actions of glucocorticoids on CDK9 recruitment, where inhibition was observed at the gene coding for IL-8 but not at NFKBIA (Luecke and Yamamoto, 2005). The up-regulation of IkB-α is part of a negative-feedback loop that limits inflammatory responses; therefore, it is unsurprising that this escapes negative regulation by IL-10 and dexamethasone.

To conclude, we provide novel insights into the mechanisms of the IL-10 antiinflammatory response. We have shown for TNF that IL-10 predominantly inhibits transcription through a unique mechanism targeting the rapid and immediate induction of transcription elongation by Pol II. IL-10 achieves this in a gene-specific manner through the inhibition of RelA-mediated recruitment of CDK9 to the TNF but not the NFKBIA promoter, thus preventing the phosphorylation of RNA Pol II at Ser2. This study, performed entirely in the physiologically relevant primary human macrophage, has
addressed long-standing contradictions within the field. The discovery of a unique negative regulatory checkpoint within the human innate immune system (CDK9 modulation of transcription elongation) has implications for the development of therapeutics that harness the central role that IL-10 plays in the suppression of inflammatory processes in humans.

MATERIALS AND METHODS

Reagents. IL-10 was a gift (Schering Plough), macrophage CSF (M-CSF) was a gift (Pfizer), and LPS was purchased (Enzo Life Sciences, Inc). Flavopiridol (Enzo Life Sciences, Inc) was used at a concentration 0.500–500.000 nm for 30 min before stimulation with LPS.

Cells. Single-donor plateletpheresis residue packs were purchased from the North London Blood Transfusion Service. Mononuclear cells were isolated by ficoll-hypeaque centrifugation (specific density, 1.077 g/ml) proceeding T cell monocyte separation in a J68 elutriator (Beckman Coulter). T cell purity was assessed by flow cytometry using directly conjugated anti-CD3 (BD), and monocyte purity, assessed using antibody and anti-CD14 antibodies (Leucogate BD), was routinely >90%. All media and sera were routinely tested for endotoxin using the limulus amebocyte lysate test (BioWhittaker Lonza) and were rejected if the endotoxin concentration exceeded 0.1 U/ml. Macrophages were derived from elutriated monocytes by culturing the cells with M-CSF at 100 ng/ml (Pfizer) in 10% heat-inactivated FCS RPMI 1640 for 3 d.

Antibodies. The anti-IκBα (C-15) antibody, anti–RNA Pol II, and anti-CDK9 (H-169) were purchased from Santa Cruz Biotechnology, Inc. Anti-tubulin was purchased from Sigma-Aldrich. Anti-lamin A/C was purchased from BD. Anti Coxl-2 was purchased from Cayman Chemical. The anti-Pol II CTD phospho-Ser2, rabbit isotype control antibody, and anti-p65 were all purchased from Abcam.

Adenoviral constructs. The TNF–luciferase reporters Adv.Luc.5′ only and Adv.Luc.5′(1037) were generated as previously described (Denys et al., 2002). The Adv.Luc.5′(785) vector was generated by PCR using the primer pairs 5′-CAGTCCTAGAGAGGACGAACATCCAACCTTC-3′ and 5′-CAGAGTCAGGAACTTTATTTGCTGGCACC-3′. The PCR product was cloned from the pcRII-TOPO TA vector using XbaI and SpeI into the 3′ region of the pAdTrack vector used to make the AdV.Luc.5′ only virus. This was used to generate the adenoviral vector by homologous recombination with the pADeasy vector in E. coli B cells and purified as per previously devised methods (He et al., 1998). Macrophages were infected with virus at a multiplicity of infection of 100 as previously described (Foxwell et al., 1998).

Nuclear extractions and Western blot analysis. After stimulation, cells were scraped into ice-cold PBS and then resuspended in hypotonic lys buffer (10 mM Hepes, pH 7.9, 10 mM KCl, 0.1 mM EDTA, 1 mM NaVO3, 0.5 mM NaF, 1 mM PMSF, protease inhibitor tablet, and 1 mM DTT). Cells were kept on ice for 15 min before the addition of NP-40 to a final concentration of 0.0064%, vortexed for 10 s, and nuclei were harvested by centrifugation (13,000 rpm for 1 min). The supernatant containing the cytosolic extract was kept at −80°C and the nuclear pellet was washed once in hypotonic lys buffer. Nuclear protein extracts were prepared by incubating the nuclei in hypertonic extraction buffer (20 mM Hepes, pH 7.9, 400 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM NaVO3, 0.5 mM NaF, 1 mM PMSF, protease inhibitor tablet [Roche], and 1 mM DTT) for 2 h with constant agitation at 4°C. Postnuclear lysates were then isolated after a 15-min spin at 13,000 rpm and frozen at −80°C. Protein assays were performed using BCA protein assay (Thermo Fischer Scientific) according to manufacturer’s instructions. Proteins were resolved by SDS-PAGE and transferred to polyvinyl difluoride membranes (Millipore), which were blocked for 1 h with blocking buffer (5% wt/vol fat-free milk and 0.1% vol/vol Tween-20 in PBS), followed by a 1-h incubation with the antibodies, which were diluted 1:1,000 in blocking buffer. Horseradish peroxidase–conjugated anti–mouse IgG or anti–rabbit IgG (GE Healthcare) were used as secondary antibodies at a dilution of 1:2,000. Bound antibody was detected using the enhanced chemiluminescence kit (GE Healthcare) and visualized using Hyperfilm MP (GE Healthcare).

Quantization of gene expression by real-time PCR. RNA was extracted from macrophages using the Blood RNA extraction kit (QiAGEN), and PCR reactions were performed and measured on a Corbett Rotor-Gene 6000 (QiAGEN) using the Superscript III platinum one-step RT-PCR kit (Invitrogen) and Assay-On-Demand premixed Taqman probe master mix (Applied Biosystems). The relative gene expression was calculated using the ΔΔCt method with the GAPDH gene for normalization of RNA levels. Primary transcript PCR was performed as follows: contaminating genomic DNA was removed from RNA samples using TURBO DNA-free kit (Applied Biosystems). The procedure routinely removed DNA contamination to levels below the detection limits of a standard real-time PCR. Reverse transcription of total RNA was performed at 42°C for 2 h with reagents purchased from Promega, followed by heat inactivation at 65°C for 10 min to inactivate the enzyme. cDNA was then subjected to real-time PCR analysis using SYBR Premix Ex Taq (Taq). The primers used for measuring primary transcript and mature transcripts were 5′-GCACTGATCAGTCTTCTCG-3′ and 5′-AGTACAGCGCCCTCT-GATGGCAC-3′ and 5′-CCTGCTGCATCTTGGATGATCG-3′ and 5′-GATACGGCCTCTGATGGCAC-3′, respectively.

ChIP. Macrophages were plated in 10-cm dishes for culture at 8.5 × 105 cells per dish. After stimulation, the cells were fixed for 10 min with formaldehyde (37% stock solution; Sigma-Aldrich) at a concentration of 1% and quenched using 125 mM Tris, pH 7.5. The nuclei were sonicated using conditions optimized for primary human macrophages. Extracts were preclreated for 2 h once with 60 μl of a 5% suspension of salmon sperm-saturated protein G (GE Healthcare) and again with another 60 μl of protein G and 2 μg ChIP-grade rabbit isotype control antibody (Abcam). Immunoprecipitations were performed at 4°C overnight. Immunocomplexes were then collected with protein G Sepharose beads for 30 min, rigorously washed, and eluted. Protein DNA cross-links were reversed by heat at 65°C for 4 h, and DNA was purified using the QIAquick PCR Purification kit (QiAGEN) and subjected to real-time PCR analysis using SYBR Premix Ex Taq. Primer pairs used for ChIP in this study were optimized and shown to amplify with similar efficiencies.

Luciferase assays. After stimulation, the amount of GFP fluorescence was measured using a FLUOstar Omega plate reader (BMG Labtech) for normalizing the levels of viral infection. Luciferase activity was measured with a Lysysys luminometer after the addition of 30 μl luciferin (Bright-Glo luciferase assay system; Promega) per well. Levels of luciferase were then normalized to the amounts of GFP.

siRNA. Macrophages were transfected with siRNA (Thermo Fisher Scientific) targeted to either RelA (RelA) or a nonspecific scrambled control (siControl), using methods previously devised and published (Behmoaras et al., 2008).

ELISA. The concentration of TNF and IL-6 in cell culture supernatants was determined by ELISA (BD), according to the manufacturer’s instructions. Absorbance was read and analyzed at 450 nm on a spectrophotometric ELISA plate reader (Labsystems Multiskan Biochromate) using Ascent software (version 2.4.2).

Online supplemental material. Fig. S1 shows that IL-10 inhibits transcription elongation at TNF. Fig. S2 shows that IL-10 requires elements 3′ and downstream of the TNF 3′ UTR for inhibition. Fig. S3 shows that IL-10 does not inhibit NF-κB activation. Fig. S4 shows that IL-10 inhibits the recruitment of RelA to proinflammatory genes. Online supplemental material is available at http://www.jem.org/cgi/content/full/jem.20100414/DC1.

This paper is dedicated to the late Professor Brian Foxwell, who was the driving force behind this work. We are grateful to Matthew Pierce who critically read this manuscript and we thank Rachel Simmonds for editorial help.
IL-10 inhibits transcription elongation of NFκB Small et al.

This work was supported by the Arthritis Research Campaign, the Kennedy Institute Trustees, and a Medical Research Council New Investigators Award.

The authors have no conflicting financial interests.

Submitted: 1 March 2010
Accepted: 4 August 2010

REFERENCES

Adelman, K., M.A. Kennedy, S. Nechaev, D.A. Gilchrist, G.W. Muse, Y. Chinenov, and I. Rogatsky. 2009. Immediate mediators of the inflammatory response are poised for gene activation through RNA polymerase II stalling. Proc. Natl. Acad. Sci. USA. 106:18207–18212. doi:10.1073/pnas.0910177106

Barboric, M., R.M. Nissen, S. Kanazawa, N. Jabran-Ferrat, and B.M. Peterlin. 2001. NF-κB/κCatp binds P-TEFB to stimulate transcriptional elongation by RNA polymerase II. Mol. Cell. 8:327–337. doi:10.1016/S1097-2765(01)00314-8

Behmoaras, J., G. Bhangal, J. Smith, K. McDonald, B. Mutch, P.C. Lai, J. Domin, L. Game, A. Salama, B.M. Foxwell, et al. 2008. fund is a determinant of macrophage activation and is associated with gomertuloporphrin synthase activity. Nat. Genet. 40:553–559. doi:10.1038/ng.137

Buelens, C., F. Willems, A. Delvaux, G. Piérard, J.P. Delville, T. Velu, and M. Goldman. 1995. Interleukin-10 differentially regulates B7-1 (CD80) and B7-2 (CD86) expression on human peripheral blood dendritic cells. Eur. J. Immunol. 25:2668–2672. doi:10.1002/eji.1830250940

Chao, S.-H., and D.H. Price. 2001. Flaviprinol inactivates P-TEFB and blocks most RNA polymerase II transcription in vivo. J. Biol. Chem. 276:31793–31799. doi:10.1074/jbc.M102306200

Denys, A.I. Udall, C. Smith, L.M. Williams, C.J. Ciociecki, J. Campbell, C. Andrews, D. Kwiatkowski, and B.M. Foxwell. 2002. Evidence for a dual mechanism for IL-10 suppression of TNF-α production that does not involve inhibition of p38: nitogen-activated protein kinase or NF-κB p50 in primary human macrophages. J. Immunol. 168:4837–4845.

Feldmann, M., and R.N. Maini. 2003. Lasker Clinical Medical Research Award. TNF defined as a therapeutic target for rheumatoid arthritis and other auto-immune diseases. Nat. Med. 9:1245–1250. doi:10.1093/nm/mrn939

Foxwell, B., K. Browne, J. Bondeson, C. Clarke, R. de Martin, F. Brennan, and M. Feldmann. 1998. Efficient adenosinal activation with IkappaB alpha reveals that macrophage tumor necrosis factor alpha activation by RNA polymerase II is NF-κB/κCatp dependent. Proc. Natl. Acad. Sci. USA. 95:8211–8215. doi:10.1073/pnas.95.14.8211

Glocke, E.-O., D. Kottlar, K. Boztug, G. Ertz, A.A. Schäffer, F. Noyan, M. Perro, J. Diestelhorst, A. Allroth, D. Murugan, et al. 2009. Inflammatory bowel disease and mutations affecting the interleukin-10 receptor. N. Engl. J. Med. 361:2033–2045. doi:10.1056/NEJMoa0907200

Gritz, G. 2005. New insights into the molecular mechanism of interleukin-10-mediated immunosuppression. J. Leukoc. Biol. 77:3–15.

Hargreaves, D.C., T. Horng, and R. Medzhidov. 2009. Control of inducible gene expression by signal-dependent transcriptional elongation. Cell. 138:129–145. doi:10.1016/j.cell.2009.05.047

He, T.C., A.B. Sparks, C. Rago, H. Hermeking, L. Zawel, L.T. da Costa, Glocker, E.-O., D. Kotlarz, K. Boztug, E.M. Gertz, A.A. Schäffer, F. Noyan, Denys, A., I.A. Udalova, I.G. Scragg, and D. Kwiatkowski. 2000. Direct evidence for involvement of NF-κB in transcriptional control of real-time gene transcription. Mol. Cell. Biol. 20:3176–3188. doi:10.1128/MCB.20.8.2629-2634.2000

Ramirez-Carrozzi, V.R., D. Bras, D.M. Bhatt, C.S. Cheng, C. Hong, K.R. Drey, J.C. Black, A. Hoffmann, M. Grez, and S.T. Shale. 2009. A unified model for the selective regulation of inducible transcription by CpG islands and nucleosome remodeling. Cell. 138:114–128. doi:10.1016/j.cell.2009.04.020

Schottelius, A.J., M.W. Mayo, R.B. Sartor, and A.S. Baldwin Jr. 1999. Interleukin-10 signaling blocks inhibitor of κCatp kinase activity and nuclear factor κCatp DNA binding. J. Biol. Chem. 274:31868–31874. doi:10.1074/jbc.274.45.31868

Takeda, K., B.E. Clausen, T. Kashiho, T. Tsujimura, N. Terada, I. Förster, and S. Akira. 1999. Enhanced Th1 activity and development of chronic enterocolitis in mice devoid of Stat3 in macrophages and neutrophils. Immunity. 10:39–49. doi:10.1016/S1074-7613(00)80005-9

Tsetsykovskaya, A.V., R. Rajkovic, J.V. Falvo, P. Legero, S.R. Neely, and A.E. Goldfeld. 2007. Activation-dependent intrachromosomal interactions formed by the TNF gene promoter and two distal enhancers. Proc. Natl. Acad. Sci. USA. 104:16850–16855. doi:10.1073/pnas.0708210104

Udalova, I.A., J.C. Knight, V. Vidal, S.A. Nedospasov, and D. Kwiatkowski. 1998. Complex NF-κB/κCatp interactions at the distal tumor necrosis factor promoter region in human monocytes. J. Biol. Chem. 273:21178–21186. doi:10.1074/jbc.273.33.21178

Udalova, I.A., V. Vidal, I.G. Szczag, and D. Kwiatkowski. 2000. Direct evidence for involvement of NF-κB/κCatp in transcriptional activation of tumor necrosis factor by a spirochetal lipoprotein. Infect. Immun. 68:5447–5449. doi:10.1128/IAI.68.9.5447-5449.2000

Wang, P., P. Wu, M.I. Siegel, R.W. Egan, and M.M. Bhillah. 1995. Interleukin (IL)-10 inhibits nuclear factor κCatp B (NF κCatp B) activation in human monocytes. IL-10 and IL-4 suppress cytokine synthesis by different mechanisms. J. Biol. Chem. 270:9588–9563. doi:10.1074/jbc.270.16.9588

Williams, L., L. Bradley, A. Smith, and B. Foxwell. 2004a. Signal transducer and activator of transcription 3 is the dominant mediator of the anti-inflammatory response in the absence of SOCS3 in macrophages. J. Immunol. 172:567–576.

Williams, L.M., G. Ricchetti, U. Sarma, T. Smallie, and B.M. Foxwell. 2004b. Interleukin-10 suppression of myeloid cell activation—a continuing puzzle. Immunology. 113:281–292. doi:10.1111/j.1365-2567.2004.01988.x

Yasukawa, H., M. Oshii, H. Mori, M. Murakami, T. Chinen, D. Aki, T. Hanada, K. Takeda, S. Akira, M. Hoshijima, et al. 2003. IL-6 induces an anti-inflammatory response in the absence of SOCS3 in macrophages. Nat. Immunol. 4:551–556. doi:10.1038/ni938

Lang, R., D. Patel, J.J. Morris, R.L. Rutschmann, and P.J. Murray. 2002. Shaping gene expression in activated and resting primary macrophages by IL-10. J. Immunol. 169:2253–2263.

Luecke, H.F., and K.R. Yamamoto. 2005. The glucocorticoid receptor blocks P-TEFB recruitment by NFκBα to effect promoter-specific transcriptional repression. Genes Dev. 19:1116–1127. doi:10.1101/gad.1297105

Murray, P.J. 2005. The primary mechanism of the IL-10–regulated anti-inflammatory response is to selectively inhibit transcription. Proc. Natl. Acad. Sci. USA. 102:8868–8869. doi:10.1073/pnas.05041910

Nakazato, G. 2009. Control of NF-κB-dependent transcriptional responses by chromatin organization. Cold Spring Harb Perspect Biol. 1:001224. doi:10.1101/cshperspect.a001224

Price, D.H. 2000. P-TEFB, a cyclin-dependent kinase controlling elongation by RNA polymerase II. Mol. Cell. Biol. 20:2629–2634. doi:10.1128/MCB.20.8.2629-2634.2000