Cutting Edge: An Inactive Chromatin Configuration at the IL-10 Locus in Human Neutrophils

Nicola Tamassia, Maili Zimmermann, Monica Castellucci, Renato Ostuni, Kirsten Bruderek, Bastian Schilling, Sven Brandau, Flavia Bazzoni, Gioacchino Natoli and Marco A. Cassatella

*J Immunol* 2013; 190:1921-1925; Prepublished online 25 January 2013;
doi: 10.4049/jimmunol.1203022
http://www.jimmunol.org/content/190/5/1921
Cutting Edge: An Inactive Chromatin Configuration at the IL-10 Locus in Human Neutrophils

Nicola Tamassia,* Maili Zimmermann,* Monica Castellucci,* Renato Ostuni,‡ Kirsten Bruderek,‡ Bastian Schilling,‡ Sven Brandau,‡ Flavia Bazzoni,* Gioacchino Natoli,* and Marco A. Cassatella*

To identify the molecular basis of IL-10 expression in human phagocytes, we evaluated the chromatin modification status at their IL-10 genomic locus. We analyzed posttranslational modifications of histones associated with genes that are active, repressed, or poised for transcriptional activation, including H3K4me3, H4Ac, H3K27Ac, and H3K4me1 marks. Differently from autologous IL-10–producing monocytes, none of the marks under evaluation was detected at the IL-10 locus of resting or activated neutrophils from healthy subjects or melanoma patients. By contrast, increased H3K4me3, H4Ac, H3K4me1, and H3K27Ac levels were detected at syntenic regions of the IL-10 locus in mouse neutrophils. Altogether, data demonstrate that human neutrophils, differently from either monocytes or mouse neutrophils, cannot switch on the IL-10 gene because its locus is in an inactive state, likely reflecting a neutrophil-specific developmental outcome. Implicitly, data also definitively settle a currently unsolved issue on the capacity of human neutrophils to produce IL-10. *The Journal of Immunology, 2013, 190: 1921–1925.

Recent data have contributed to expand the view of polymorphonuclear neutrophils as versatile cells for their capacity to cross talk with cellular components of the innate and adaptive immune systems, as well as for their ability to condition the evolution of various processes via the release of newly synthesized cytokines (1). Accordingly, the cytokine repertoire that neutrophils can potentially express is quite vast and includes proinflammatory/anti-inflammatory and immunoregulatory cytokines, chemokines, TNF superfamily members, colony-stimulating, and angiogenic factors (2). Interestingly, although differences in the capacity to express cytokines have been reported to occur between human and mouse neutrophils, there is not a general consensus in the literature whether human neutrophils produce IFN-γ, IL-6, IL-17, or IL-10 (1). With regard to IL-10, our groups and others have recently demonstrated that highly purified neutrophil populations, isolated by a variety of procedures from healthy donors, do not express or secrete IL-10, either spontaneously or upon treatment with a panel of stimuli (including serum amyloid protein A [SAA], LPS, Pam3CSK4, polynosinic:polycytidylic acid, R-848, curdlan, neutrophil-activating protein derived from Helicobacter pylori, the chemooattractant fMLF, insoluble immunocomplexes, IFN-γ, TNF-α, GM-CSF, or G-CSF) used singly and in combination (3). In contrast, we confirmed (4) that autologous monocytes, stimulated under the same experimental conditions as neutrophils, promptly produce detectable amounts IL-10 (3). Our data were in line with analogous observations previously made in several laboratories (5–9), but in contradiction with findings reported by other researchers, who had instead observed a neutrophil-derived IL-10 production under resting or stimulatory conditions (10–14). In particular, the study by De Santo et al. (14), showing that SAA-1 induces the differentiation of IL-10–secreting neutrophils with potential immunosuppressive activities in melanoma patients, has instigated great interest for its implications in the cancer setting. In contrast, there is no doubt that mouse neutrophils do produce IL-10, as reproducibly observed either in vitro experiments (3) or under a variety of experimental models (1), including during methicillin-resistant Staphylococcus aureus infection (15), pneumonia (16), and polymicrobial sepsis (17). Because of the importance of IL-10 either in controlling degree and duration of inflammatory reactions (18), or in promoting immunosuppression in tumors (18), a molecular understanding of how the expression of this cytokine is differentially regulated in different cell types has obvious implications not only for a better comprehension of inflammatory disease pathogenesis, but also for immunotherapeutic strategies in cancer patients.

Changes in chromatin organization and posttranslational modifications control the transcriptional outputs in response to...
specific environmental conditions or developmental states (19, 20). Covalent modifications of histones are considered to be involved in regulating chromatin organization and in positive or negative control of gene expression occurring during normal embryonic development (21), cancer (22), and the evolution of specific acquired immune responses (23). Among a wide range of posttranscriptional modifications, methylation and acetylation of specific histone residues are pivotal in the maintenance of the expression of a given gene in an active or suppressed state (24, 25). Accordingly, methylation of histone H3 at K9 and K27, and histone H4 at K20 (H3K9, H3K27, and H4K20) are correlated with repression of transcription, whereas methylation of histone H3 at K4, K36, and K79 (H3K4, H3K36, and H3K79) is implicated in activation of transcription. Histone posttranslational modifications can be detected by chromatin immunoprecipitation (ChIP) using specific Abs for modified histones. ChIP has been instrumental not only to clarify that histone modifications are often dynamic, as well as reversible, but also to establish combinations of marks associated to different states of gene activity (26). Active promoters are associated with trimethylated histone H3 at K4 (H3K4me3), which usually co-occurs with different levels of acetylated histone H3 or H4 (AcH3 and AcH4), depending on the state of gene activity. Enhancers are marked by H3K4me1 and can be divided into poised or active based on the absence or presence of acetylated histone, respectively (19, 20). Conversely, trimethylation of K27 of histone 3 (H3K27me3) represents a mark for silent genomic regions (27).

In this study, we asked whether the different ability of human neutrophils and monocytes to activate IL-10 expression might reflect a different basal chromatin organization of the human IL-10 locus, which would, in turn, depend on the action of highly specific developmental inputs, selectively acting in either cell type (28).

Materials and Methods

Cell purification and culture

Granulocytes were isolated from buffy coats of healthy volunteers by Ficoll-Paque gradient followed by dextran sedimentation and hypotonic lysis of erythrocytes, under endotoxin-free conditions (29). Neutrophils were then enriched to reach purity of >99% by positively removing any contaminating cells from granulocytes, using the EasySep neutrophil enrichment kit (Stem Cell Technologies) (3). Human monocytes were purified from PBMCs with anti-CD14 microbeads (>98%; Milteny). In selected experiment, neutrophils were isolated from the peripheral blood of melanoma patients (all stage IV M1c) or healthy volunteers. In the latter case, diluted blood was subjected to density gradient centrifugation (LSM 1077-Lymphocyte Separation medium; PAA Laboratories GmbH), and neutrophils (purity > 98%) were then isolated by sedimentation over 1% polyvinyl alcohol by hypotonic lysis (0.2% NaCl) of erythrocytes (3). Mouse neutrophils were isolated from bone marrow of C57BL/6 mice, using a discontinuous Percoll gradient according to standard procedures (30), and further purified by positive selection using anti-Ly-6G Ab conjugated with allophycocyanin (Milteny) and anti-allophycocyanin MicroBeads (Milteny). Immediately after purification, human or mouse cells were either subjected to lysis for ChIP assays or suspended in RPMI 1640 medium supplemented with 10% low-endotoxin FBS (<0.5 endotoxin unit/ml; BioWhittaker), treated or not with 100 ng/ml ultrapure Escherichia coli LPS (0111:B4; Alexis), 5 μg/ml ApoSA (500–13; PeproTech), or 1 μg/ml Pam3CSK4 (Invivogen), and then cultured at 37°C, 5% CO2 atmosphere for up to 6 h. Human samples were obtained following healthy donors’ and patients’ informed written consent. The study has been cleared by the Ethic Committees of both the Azienda Ospedaliera Universitaria Integrata di Verona (Italy) and the University Hospital Essen (Germany).

Quantitative real-time RT-PCR

Primary transcript (PT) quantitative RT-PCR analyses were performed as previously described previously (31), using gene-specific primer pairs (Invitrogen) available in the public database RTPrimoDB (http://medgen.ugent. be/rprimed), under the following entry codes: PT–IL-10 (8612), PT–TNF-α (8613), and GAPDH (3539). Data were calculated by Q-Gene software (http://www.gene-quantification.de/download.html) and expressed as mean normalized expression units after GAPDH normalization.

ChIP assays

ChIP experiments were performed as described elsewhere (31), with minor modifications. In brief, nuclear extracts were prepared from 1 × 106 cells (depending on the type of ChIP assay) and then sonicated and immunoprecipitated with mAbs recognizing H3K4me3 (Millipore) or polyclonal Abs recognizing H4Ac, H3K27me3, H3K9me3, and SP1 (Millipore), H3K4me1 and H3K27Ac (Abcam), and C/EBPβ, C-FOS, and NF-kB p50 (Santa Cruz). The coimmunoprecipitated material was then subjected to quantitative PCR analysis using specific primers (purchased from Invitrogen), either spanning the human IL-10 locus or the murine IL-10 locus. Sequences of the ChIP primers used are available on request. The various histone modifications were quantified also at the promoter of prolactin because it is completely silent in myeloid cells to establish the background levels of ChIP experiments.

Results and Discussion

Chromatin organization of the IL-10 locus in human neutrophils and monocytes

To elucidate whether cell-specific histone modification signatures might provide indicative elements for the differential expression of IL-10 in human phagocytes, we initially performed a comparative analysis of the chromatin status at the IL-10 locus in human neutrophils and autologous monocytes freshly isolated from the peripheral blood. Neither induction of IL-10 gene expression (data not shown) nor RNA polymerase II recruitment at the IL-10 promoter (data not shown) were detected in neutrophils stimulated with 100 ng/ml LPS or 5 μg/ml SAA. We thus scanned 20 kb of the IL-10 genomic locus (spanning from −15.0 to +7.5 kb relative to the transcription start site; Fig. 1A) for the presence of chromatin modifications associated with transcriptionally active genes (20), including: 1) histone H3 trimethylated at K4 (H3K4me3), which is localized at a few nucleosomes surrounding the transcription start site and strongly correlates with active transcription (32); 2) tetraacetylated histone H4 (H4Ac) and histone H3 acetylated at K27 (H3K27Ac), which are enriched in active euchromatin (33); and 3) histone H3 monomethylated at K4 (H3K4me1), which is detected at both promoters (in association with H3K4me3) and enhancers (34). In addition, we tested the levels of marks that are negatively correlated with gene expression, including H3K9me3, H3K27me3, and DNA methylation (19).

Neither H3K4me3 (Fig. 1B) nor acetylated histones (Fig. 1C) were detectable at the IL-10 locus of freshly isolated neutrophils, whereas they were both present in autologous monocytes (Fig. 1B, 1C). Moreover, no association of H3K27Ac was found at the IL-10 genomic locus of neutrophils (Fig. 1D), whereas H3K4me1 was present, even though at much lower levels than in monocytes (Fig. 1E). Interestingly, H3K9me3, H3K27me3 (data not shown), and DNA methylation (data not shown) were all undetectable at the IL-10 genomic locus of either neutrophils or monocytes, thus ruling out transcriptional repression mediated by these marks (20). Taken together, data are in keeping with the notion that the IL-10 genomic locus is in an inactive state in human neutrophils, whereas it is poised for activation in monocytes (20).

Chromatin organization of the IL-10 locus in activated neutrophils and monocytes

Because induction of inflammatory gene expression is associated with chromatin remodeling and changes in histone
modified (35), we examined chromatin marks in neutrophils and monocytes stimulated with LPS, SAA, or Pam3CSK4 (Supplemental Fig. 1). Although activation marks (especially H3K4me3 and H3K27Ac) were all upregulated in activated monocytes, no substantial changes were observed in activated neutrophils (Supplemental Fig. 1). Similar results for IL-10 promoter and intragenic regions amplified by primer sets 5 and 8 (as depicted in Fig. 1A) were observed in neutrophils and monocytes, either cultured as early as for up to 5 h (data not shown) or stimulated with 500 μg/ml zymosan, 100 μg/ml β-glucan or β-glucan plus LPS (data not shown). Conversely, H3K4me3, H4Ac, H3K4me1, and H3K27Ac were all detected at the CXCL8 gene promoter in neutrophils, being (with the exception of H3K4me1) further increased upon LPS, SAA, or Pam3CSK4 stimulation, even at relatively higher levels than in monocytes (Supplemental Fig. 1). Furthermore, ChIP of a number of transcription factors (TFs), previously proposed to bind to and/or transactivate the IL-10 gene in various cells of human or mouse origin (36, 37), revealed no binding of C/EBPβ (Fig. 2A, left graph), c-FOS (Fig. 2B, left graph), SP1 (Fig. 2C, left graph), and NF-κBp50 (Fig. 2D, left graph) to the IL-10 promoter of neutrophils, either constitutively or upon activation with LPS. The same TFs, however, were found to bind to neutrophil promoters of other known target genes (1) (Fig. 2, right graphs). Altogether, data are all consistent with an inactive state of the IL-10 genomic locus in human neutrophils.

**Chromatin organization of the IL-10 locus in neutrophils isolated from melanoma patients**

Subsequently, we examined chromatin marks also in melanoma neutrophils, because they were reported to produce dramatically high amounts of IL-10 (14), a finding that we failed to reproduce (3). We observed that, similar to neutrophils of healthy subjects, neutrophils isolated from stage IV melanoma patients, and cultured without or with SAA or LPS, did not exhibit any H3K4me3 and H4Ac marks (Fig. 3) at regions of the IL-10 locus that showed very high signals in monocytes. The latter observations suggest that no chromatin...
reorganization occurs at the IL-10 genomic locus of melanoma neutrophils.

Chromatin organization of the IL-10 locus in mouse neutrophils

Finally, we measured chromatin marks and TF binding also in mouse neutrophils (Supplemental Fig. 2). As a result, we detected increased levels of H3K4me3, H4Ac, H3K4me1, and H3K27Ac at synergetic regions of the IL-10 locus of unstimulated cells (Supplemental Fig. 2A–E), as well as a detectable C/EBPβ, c-FOS, SP1, and NF-κBp50, binding to the IL-10 locus upon cell activation with LPS (Supplemental Fig. 2F–I), thus in full agreement with the well-known potential capacity of mouse neutrophils to express and release IL-10 (3, 15, 16, 38). All in all, our experiments clearly suggest that different regulatory circuits and lineage-specific TFs control the state of the IL-10 locus in human and mouse neutrophils.

In conclusion, overall, data shown in this article are consistent with the notion that human neutrophils, either from blood of healthy subjects or from melanoma samples, cannot produce IL-10 upon activation. Because the contrasting results related to IL-10 expression by human neutrophils (5–14) were not explained by different experimental settings, peculiar purification protocols, or cell desensitization (3), the results shown in this article indicate that lack of IL-10 induction in human neutrophils correlates with an inactive and neutrophil-specific basal chromatin organization. Accordingly, our data indicate that chromatin at the IL-10 genomic locus is differentially organized in human neutrophils and monocytes, and only in the latter it appears to be poised for transcription in response to stimulation. Therefore, basal differences in the chromatin state of the IL-10 locus, rather than a different responsiveness to activating signals, likely account for the differential ability of neutrophils and monocytes to switch on transcription of this specific gene. Although we cannot exclude that yet-undefined situations might provoke major reorganization of the IL-10 genomic locus of human neutrophils, thus making it possible to activation, our current results clearly exclude that human neutrophils can express IL-10 under standard conditions. Consequently, because it has been reported that interaction of neutrophils with invariant NK T cells would result in less neutrophil-derived IL-10, a phenomenon supposed to have major influences on the disease outcome in melanoma patients (14), our current data prompt for a reevaluation of planning to target presumed immunosuppressive, IL-10–producing neutrophils (14).

Acknowledgments

We are grateful to Federica Calzetti and Elena Caveggion for expert technical assistance.

Disclosures

The authors have no financial conflicts of interest.

References


Downloaded from http://www.jimmunol.org/ by guest on April 13, 2017


AAI Public Affairs ONLINE

Visit us to
- Learn about NIH funding
- Keep current on key policy issues
- Discover how you can help AAI in its advocacy initiatives

Go to www.aai.org and click on Public Affairs.

GRIP
Grant Review for Immunologists Program

Get a GRIP: An AAI program designed to help new investigators prepare their NIH grant proposals

AAI is pleased to offer a program to match new PIs with established PIs who have significant, successful grant writing careers. The Grant Review for Immunologists Program (GRIP) invites new PIs to submit an outline or NIH-style abstract to the GRIP coordinator who, with the assistance of a small volunteer subcommittee, will attempt to match the topic of the proposal with the research experience of an established PI. Matches will be made as quickly as possible to allow new PIs to meet upcoming NIH grant deadlines. Participation is open only to AAI members and is strictly voluntary. The program is not intended to supplant internal mentoring programs.

GRIP is now accepting both new PI and established PI participants. Please send your CV and a brief description of either your potential research project (new PIs) or grant reviewing experience (established PIs) to info@aaai.org (please write “GRIP” in the subject line).

Program details at www.aai.org/GRIP_rd.htm