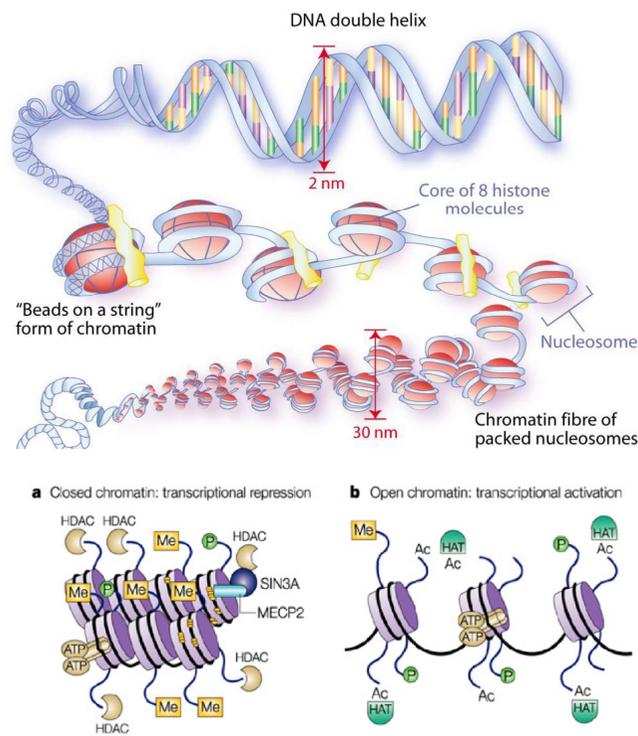


INTRODUCTION

More than 2 m of DNA is packaged into the nucleus of every single human cell as chromatin. The fundamental subunit of chromatin is the nucleosome. Each nucleosome consists of approximately 150 base pairs of negatively charged DNA wrapped around a positively charged histone octamer “spool.” Histones may be modified by the addition of different chemical functional groups (acetyl groups, methyl groups, ubiquityl groups, etc.). These “covalent modifications” of histone proteins affect the interactions between DNA and histones, thus altering gene expression and organization of the chromosomes. Histone acetylation has been shown to increase chromatin accessibility by altering the positions and density of nucleosomes. Histone acetyltransferases (HATs) catalyze the transfer of acetyl groups to histones, and histone deacetylases (HDACs) catalyze the reverse reaction. Over the past decade small-molecule inhibitors of HDACs (HDAC inhibitors, called HDACis) have been used as antiproliferative and anti-inflammatory therapies in malignancies. Butyrate, the natural product of the bacterial fermentation in the small bowel of dietary fiber that has escaped digestion, is an HDACi with anti-inflammatory and antineoplastic effects. Elucidation of the mechanism of action of this important natural-product, anti-inflammatory, potential anticancer HDACi is badly needed. We have correlated the changes gene expression and with changes in chromatin structure of several human cell lines before and after treatment with butyrate. By monitoring these “epigenetic” changes in nucleosome position and chromatin structure resulting from butyrate treatment, we have gained a mechanistic understanding of gene regulation and chromatin structure and their relation to inflammation.



METHODS AND RESULTS

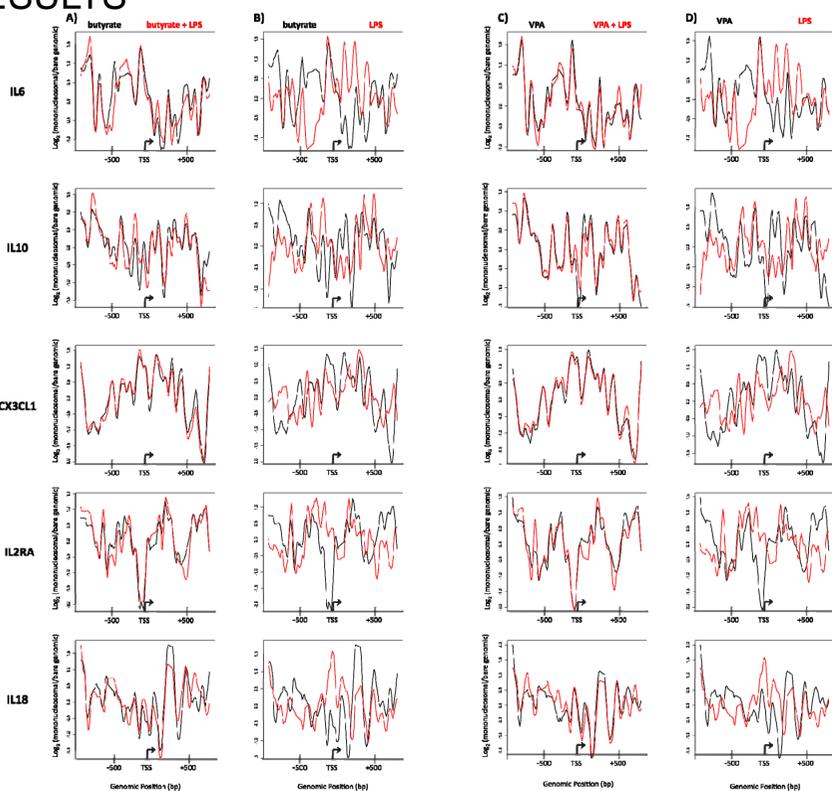


Fig. 2 Log₂(mononucleosomal DNA/bare genomic) plotted for each of 5 genes 2000bp centered around the TSS. A) butyrate (black) and butyrate pretreatment followed by LPS (red). B) butyrate treatment alone (black) and LPS stimulation only (red). C) valproic acid treatment (black) and valproic acid pretreatment followed by LPS (red). D) Valproic acid treatment (black) and LPS stimulation only (red).

To assess the effects that HDAC inhibitor have on chromatin structure during inflammation, we compared human macrophages (phorbol-12-meristate-13-acetate (PMA) differentiated U937 monocytic cells) stimulated with lipopolysaccharide (LPS) (200ng/ml) for 4h and macrophages pretreated with HDAC inhibitor and then stimulated with LPS. The pretreatment consisted of 2mM butyrate or 1mM valproic acid for 1h. We monitored nucleosome distribution with an innovative use of whole gene tiling microarrays allowing us to generate highly sensitive plots of nucleosomal distribution across the promoter regions of 505 immunity related genes. Herein we show that HDAC inhibitor treatment alone induces modest changes in chromatin structure at the transcription start sites of inflammatory genes compared with the chromatin structure observed in resting macrophages. Contrarily, extensive changes were observed at over 90% of the genes studied in macrophages stimulated with LPS. Moreover, our results indicate that HDAC inhibitor treatment restricts changes in chromatin structure during an inflammatory insult, suggesting that HDAC inhibitor “lock-in” chromatin structural states at inflammatory-related genes in macrophages. In addition, we have preliminary data suggesting that the locking of the chromatin structure is associated with lack of genes expression. These data suggest that the anti-inflammatory mechanisms of HDAC inhibitors involve regulation of chromatin structure and suggest a critical role of epigenetic mechanisms during inflammation.

SIGNIFICANCE

IL-6 shows HDACi regulated chromatin structural changes. IL-6 has many functions, including proliferation of B-cells and activating acute phase proteins³⁰. Acute phase proteins are key molecules in regulating the immune response. Patients with inflammatory diseases, such as rheumatoid arthritis and inflammatory bowel disease have an upregulated expression of IL-6. It has also been shown that IL-6 and acute phase proteins serve as effective markers of severity of these diseases, where an increase in IL-6 or acute phase proteins mark a more severe disease state. Moreover, there are therapeutic approaches that down regulate IL-6 production to assist in reducing the inflammation that these patients experience. This is significant as HDAC inhibitors to dramatically decrease IL-6 protein expression. Importantly, we have linked the anti-inflammatory properties of HDAC inhibitors to the alterations in the chromatin regulatory structural potentials. By blocking the nucleosomal redistributions that occur within TSS induced during an immune response, these class 1 HDAC inhibitors have the potential to regulate key players in the inflammatory response and prevent them from being constitutively expressed. These results are very important in light of the fact that butyrate is a front-line drug in IBD therapy. This is the first report linking the chromatin structural regulatory abilities of histone deacetylase inhibition and its anti-inflammatory effects. Herein we provide the first insights into the promoter structures associated with hundreds of immunity and inflammation related genes in response to an immune stimulus. We demonstrate that the anti-inflammatory mechanisms of HDAC inhibitors are likely a result of the of chromatin stabilizing properties during an immune insult. Further studies of this nature will help to illuminate the pathology of immune diseases and guide the identification of effective and targeted treatments.

