Dendritic cells at the interface of innate and acquired immunity: the role for epigenetic changes

Haitao Wen,* Matthew A. Schaller,* Yali Dou,*,† Cory M. Hogaboam,* and Steven L. Kunkel*,[1]

*Departments of *Pathology and †Biological Chemistry, University of Michigan Medical School, Ann Arbor, Michigan, USA*

Abstract: Dendritic cells (DC) are known to be essential immune cells in innate immunity and in the initiation of adaptive immunity. The shaping of adaptive immunity by innate immunity is dependent on DC unique cellular functions and DC-derived effector molecules such as cytokines and chemokines. Thus, it is not surprising that numerous studies have identified alterations in DC number, function, and subset ratios in various diseases, such as infections, cancers, and autoimmune diseases. Recent evidence has also identified that immunosuppression occurring after severe systemic inflammation, such as found in sepsis, is a result of depletion in DC numbers and a later dysfunction in DC activity. This correlation suggests that the sustained DC dysfunction initiated by life-threatening inflammation may contribute to the subsequent immunoparalysis, potentially as a result of the long-term maintenance of an abnormal gene expression pattern. In this review, we summarized the present information regarding altered DC function after a severe, acute inflammatory response and propose a mechanism, whereby epigenetic changes can influence long-term gene expression patterns by DC, thus supporting an immunosuppression phenotype. *J. Leukoc. Biol.* 83: 439–446; 2008.

Key Words: *sepsis · cytokine · Toll-like receptors · host defense · cell memory · long-term*

INTRODUCTION

Since the first description by Steinman's group as a novel cell population in the mouse spleen [1–3], dendritic cells (DC) have been recognized as a key component to innate immunity and in the initiation of an adaptive immune response [4]. DC are relatively rare cells and are mainly localized in tissues exposed to external environments, such as the respiratory system and gastrointestinal tract, where they reside in an immature form and serve as sentinels. Immature DC express a number of pathogen recognition receptors (PRRs), including TLRs, c-type lectin receptors, and nucleotide-binding oligomerization domain-like receptors, which aim to detect pathogen-associated molecule patterns. DC also participate in cell-mediated immunity by taking up antigen and incompletely degrade them into peptides optimal for presentation by relevant MHC and CD1 molecules [5]. The activation of DC by pathogen-derived molecules induces a switch in chemokine receptor expression and subsequent migration to draining lymph nodes in a chemokine receptor-dependent manner. The up-regulation of costimulatory (CD80, CD86, CD40) and MHC molecules, in conjunction with secretion of cytokine and chemokines, facilitates DC antigen presentation to naïve T cells with an antigen-specific receptor [6].

Given their essential role in the immune response, any alteration in DC number and/or function would impair normal immune activity and subsequently render the host susceptibility to secondary pathological challenges. Interestingly, a number of clinically important diseases have been identified that target the DC and reduce their ability to fully function in host defense. For example, infection of DC with measles suppresses their allostimulatory properties for $CD4^+$ T cells [7]. Systemic activation of DC by TLR agonists or infection with a malaria parasite (*Plasmodium berghei*) impairs their cross-presentation and antiviral functions [8]. Tumors may secrete regulatory cytokines such as IL-10, TGF-β, and vascular endothelial growth factor and reduce DC development and function, resulting in attenuated expression levels of CD80 and CD86 on tumor-infiltrating DC [9]. Furthermore, tumors can convert immature DC into TGF-β-secreting cells, which subsequently induce the proliferation of regulatory T cells [10] and enhance the production of Th2 cytokines (IL-4 and IL-13) by naïve $CD4^+$ T cells [11].

One of the best examples of DC dysfunction associated with a disease state is presented by studies investigating the molecular and cellular mechanisms of severe sepsis in patients and experimental models. It is clear that patients who survive severe sepsis are more susceptible to a variety of diseases long after being discharged from the intensive care unit [12, 13]. In an experimental study of severe sepsis, cecal ligation and puncture (CLP) has been proposed to replicate the nature and course of polymicrobial-induced clinical sepsis [14, 15]. The benefits of the CLP model are its reproducibility and the potential to alter the severity of sepsis by controlling needle size, number of cecal punctures, and antibiotics use [16]. Our

[1] Correspondence: Department of Pathology, University of Michigan Medical School, 4071 BSRB, 109 Zina Pitcher Place, Ann Arbor, MI 48109-2200, USA. E-mail: slkunkel@umich.edu

Received June 8, 2007; revised September 26, 2007; accepted October 20, 2007.

doi: 10.1189/jlb.0607357

CLP procedure results in ~40% mortality in the acute phase of sepsis [17–19]. Following the induction of CLP-induced peritonitis, inflammatory cytokines/chemokines such as IL-12p70, IL-10, TNF-α, CCL2, CCL3, and CXCL10 are rapidly induced in the peritoneal cavity (local response; **Fig. 1A**) and blood (Fig. 1B) within 4 h and peak at 24 h. Three days later, the local and systemic levels of inflammatory cytokines/chemokines mostly return to baseline levels, indicating the end of the acute phase of sepsis. It has been demonstrated that animals surviving septic peritonitis induced by CLP were more susceptible to a secondary fungal challenge [17]. Thus, clinical reports and experimental animal studies conclude that an enigmatic complication of severe sepsis is a lasting, immunosuppressive state. While the molecular and cellular mechanisms that support this immunoregulation remain elusive, it is known that rapid DC depletion as a result of extensive apoptosis following the acute phase of severe sepsis is a contributing factor [20]. Impairment in DC functions, including cytokine production and allogeneic T cell activation, has also been reported, although the underlying, molecular mechanism for these altered responses is not clear [18, 21].

Recent data support the concept that specific gene expression patterns, which determine the behavior and property of individual cells, are under the control of epigenetic alterations. These effects are exerted via a variety of chemical modifications to chromatin structure, including DNA methylation, histone modification, chromatin remodeling, and histone replacement, and have been studied extensively in normal embryogenesis and cancer [22–24]. Although the exact molecular mechanism(s) is still under intensive investigation [25, 26], it is accepted that epigenetic alterations can be transmitted from generation to generation and carry heritable information that is not coded by genomic DNA. The long-term alteration in DC functions, which correlates with sustained immunosuppression following severe sepsis, supports the concept for a potential epigenetic-dependent mechanism in this process.

DC IN SEPTIC RESPONSE

There is little doubt that DC, although small in number, play a large role in maintaining an appropriate and protective innate and acquired host response. Therefore, DC are logical participants in the initial severe infection and dysregulated cytokine storm in the early stage of severe sepsis. Indeed, DC undergo a rapid depletion in septic patients [27] and in experimental sepsis animal models [21, 28–31]. Thus, severe sepsis causes the depletion of DC, a potent APC, which subsequently compromises the innate and adaptive immune response [20]. A kinetic analysis of DC subtypes following the induction of experimental peritonitis identified a depletion of myeloid DC and plasmacytoid DC (pDC) in secondary lymphoid organs (spleen and lymph node) and peripheral organ (lung). Interestingly, the DC populations are reseeded to the distant organs, as their numbers gradually return to normal levels after a significant recovery period (H. Wen and S. L. Kunkel, unpublished data). It remains unclear where the generation of DC subtypes from their precursor occurs post-sepsis. It is possible that DC are newly generated in bone marrow and migrate to peripheral sites via the action of chemokines [32]. Another possibility is that DC are generated in peripheral sites from precursor cells, which have been recruited into the periphery during the severe inflammatory response. It is important to answer these questions, as normal hematopoiesis is influenced by the surrounding tissue microenvironment (hematopoietic niche) [33, 34]. The tissue location in which DC are generated will definitely contribute to the final DC phenotypes and direct the properties of newly generated DC.

Based on the long-term susceptibility of septic patients to secondary pathological infections [35] and our observation of a gradual restoration of DC number after severe sepsis, it is highly possible that sepsis-induced depletion of immune cells is not the only mechanism responsible for the long-term immunosuppression present in post-septic patients and animals. Alterations of normal immune cell functions could be another potential mechanism leading to an inappropriate immune response. It is worth noting that apoptosis and dysfunction of immune cells are not exclusive to each other. It has been reported that apoptotic cells induce anergy and a Th2 response in surviving immune cells [20, 36, 37], promote the production of anti-inflammatory cytokines by DC and macrophage [38], and inhibit the release of proinflammatory cytokines [39, 40]. In contrast to the convincing data showing sepsis-induced

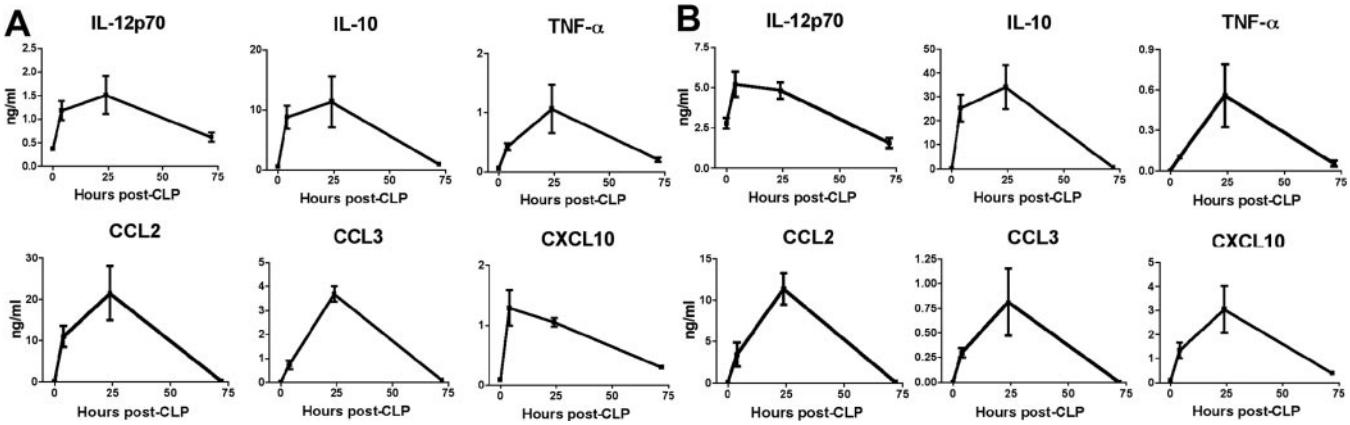

Fig. 1. Cytokine profile following experimental peritonitis. At different time-points (4, 24, and 72 h) after the CLP procedure, peritoneal lavage fluid (A) and blood samples (B) were collected. Levels of inflammatory cytokines were determined by ELISA.

depletion of immune cells, few investigations have focused on alteration in immune cell function after sepsis. Previously, our group has characterized the immunosuppressive status of the survivors of severe experimental sepsis. These post-septic mice were susceptible to a pulmonary challenge of *Aspergillus fumigatus* compared with sham-operated mice, highlighting impaired pulmonary immunity in post-septic mice. *A. fumigatus* was chosen as the microbial challenge, as this fungus normally is not a pathogen for an intact pulmonary immune system [17, 41]. Furthermore, adoptive transfer of bone marrow-derived DC from sham mice restored the protective immune response of post-septic mice to *A. fumigatus* challenge. These observations led us to hypothesize that severe sepsis-induced DC dysfunction is an important mechanism of long-term immunosuppression after the acute phase of sepsis.

CHRONIC CONSEQUENCES OF SEVERE SEPSIS

General consensus supports the idea that the acute inflammatory response is intimately linked to innate immunity, which usually occurs over a relatively short period. During this limited time-frame, a functional, acute inflammatory response is supported by the temporal expression of a set of inflammatory mediators and activation of specific leukocyte subpopulations. For example, within the first hour of in vivo LPS challenge, mRNA expression for early response cytokines such as TNF-α can be detected in mononuclear phagocytic cells, followed by the expression of secondary response cytokines and chemokines [42, 43]. In animal experiments, this acute cytokine phase is followed by the recruitment of neutrophils, which occurs within hours [44]. Over the subsequent 72 h, cytokine, chemokine, and leukocyte levels will usually subside, and any alteration in local tissue will begin to be repaired [44].

Interestingly, this scenario is dependent on the quantity and complexity of the initial antigen or pathogen [44, 45]. An increase in the amount of stimuli will result in an enhanced cytokine and leukocyte response, and an increase in the complexity of the challenge may lead to a prolonged reaction. An additional factor that determines the clinical course of an acute inflammatory response is the rigor of the host's immune response to the inciting agent [35, 46]. This aspect of the response has important consequences for the short-term course of the acute reaction and also appears to play a key role regarding the host's response to a subsequent challenge that occurs long after the initial severe acute reaction. An interesting inverse clinical correlation exists between the severity of the initial acute inflammatory response and the ability of the host to deal with a subsequent immune challenge months later [35, 47, 48].

As noted above, one of the best examples of the chronic effects of severe acute inflammation is presented by data modeled from human sepsis. A variety of investigations has studied the short-term, less-than-30-days sequela of the septic response and has reported mortalities that approach 50% [13]. Additional investigations have noted that the septic population is at a significant risk of dying for many years after the initial hospitalization [35]. Septic patients develop a sustained anti-inflammatory or immunosuppressive state that has been termed "immunoparalysis" following the initial hyperinflammatory response, which is manifested by an inability to eradicate the primary infection and/or the development of new secondary infections [12, 13, 41, 49–51]. Many of the pathogens responsible for the secondary, hospital-acquired infections are not particularly pathological in patients with competent immune systems, indicating the immunosuppressive state of patients with sepsis. Thus, the initial episode of severe sepsis, characterized by a dysregulated, inflammatory response, leads to long-lasting complications regarding how the host responds to and deals with subsequent challenges. The chronic consequences linked to severe acute inflammation are not limited to sepsis but are found in other severe acute human diseases, including the long-term consequences of acute ischemia/reperfusion injury post-organ transplant [52], post-recovery alterations from severe respiratory syncial viral infection in neonates [53, 54], and the chronic consequences observed in response to severe burn and trauma injury [55, 56]. Many of these clinical cases appear to be governed by severe, life-threatening, inflammatory events, followed by a long-lasting, improper or immunosuppressed immune response.

EPIGENETIC MECHANISM OF GENE REGULATION

Epigenetics is the study of mitotically and/or meiotically heritable changes in gene function that cannot be explained by changes in DNA sequence. These changes include DNA methylation, histone modification, chromatin remodeling, and histone variant incorporation [57]. In the last decade, biologists have convincingly demonstrated that the regulation of chromatin structure or epigenetic regulation confers heritable stabilization of gene expression patterns and thereby, plays an essential role in basic physiological processes and in diseases [22, 58]. In addition to interactions between transcription factors and DNA sequences in promoters and other regulatory regions, gene expression is influenced by epigenetic changes in chromatin structure that control the access of DNA-binding proteins to their conserved sites during the initiation period of transcription. Conversely, transcription factors can dictate epigenetic changes by recruiting histone-modifying enzymes and chromatin-remodeling complexes.

In eukaryotic cells, DNA is packed into a high-order nuclear structure, called chromatin, with the assistance of chromosomal proteins, including histones (**Fig. 2**). The basic unit of chromatin is the nucleosome, which consists of 146 bp of double-strand DNA wrapped around a histone octamer protein core (two molecules each of H2A, H2B, H3, and H4) [59, 60]. At present, histone modifications, including acetylation, phosphorylation, methylation, and ubiquitylation, are under intensive investigation and have been considered to be important epigenetic mechanisms regulating gene expression. These modifications, catalyzed by several families of histone-modifying enzymes, form a "histone code" that constitutes an important epigenetic determinant of the transcriptional state [25, 61]. Individual histone modifications can be simply categorized as "permissive" or "repressive", in reference to gene transcriptional activity. For example, histone acetylation is generally

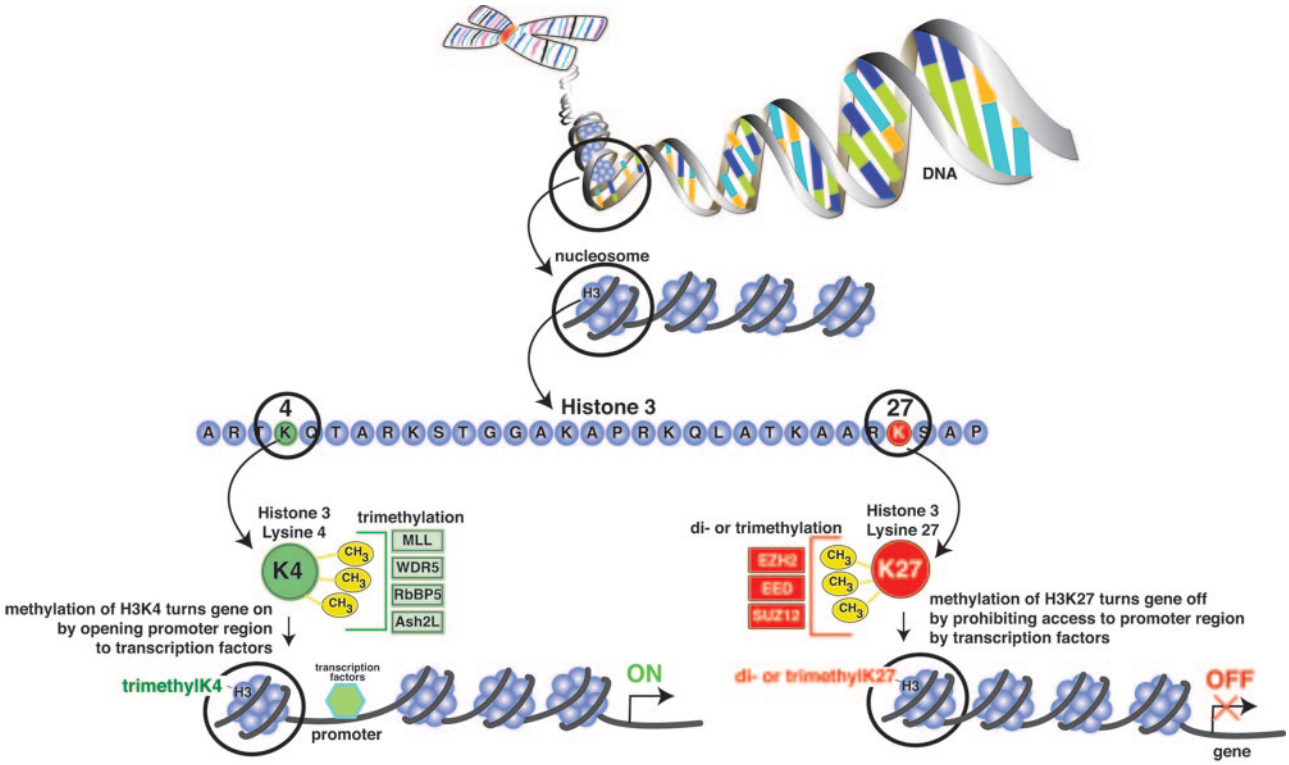

Fig. 2. Diagram of the biochemical effects of methylation at histone H3 lysine-27 (H3K27), which maintains the chromatin in a conformation that does not allow transcription factors access to a promoter, and the gene is silenced. When H3K4 is methylated, the conformation of the chromatin is relaxed, and the transcription factors now have access to the promoter, resulting in gene expression. Mixed lineage leukemia (MLL) complex, containing core components MLL, WDR5, RbBP5, and Ash2L, possesses methyltransferase enzyme activity specific for H3K4, while Polycomb repressive complex 2 (PRC2), containing embryonic ectoderm development (EED), enhancer of Zeste 2 (EZH2), and suppressor of Zeste 12 (SUZ12), specifically mediates H3K27 methylation.

associated with gene activation, partially as a result of neutralization of the positive charge on lysine and a consequent lowered stability of interactions between histones and the negatively charged DNA [62].

In addition, histone methylation is recognized as an important modification linked to transcriptional activation and repression [61]. As the first observation of histone methylation was to regulate RNA synthesis, Jenuwein's group in 2000 [63] made a landmark discovery of molecular identity of the first histone methyltransferase (HMT), SUV39H1. Histones contain numerous lysine and arginine residues and can be modified on both residues with up to three methyl groups. Methylation of lysine residues at six sites (H3K4, -9, -27, -36, and -79 and H4K20) has been linked to transcriptional regulation [61, 64], which in conjunction with various methylation levels (mono-, di-, or trimethylation), provides remarkable regulatory potentials for chromatin modification and gene regulation.

Lysine methylation is mediated by the Su(var)3-9, Enhancer of Zeste, Trithorax (SET) domain, which was initially characterized as a common motif in three main classes of HMTs in *Drosophila*: SU(VAR)3-9 [65], the *Polycomb*-group protein E(Z) [66], and trithorax protein [67]. They are specifically responsible for the methylation of H3K9, H3K27, and H3K4, respectively. H3K4 methylation (H3K4me) generally correlates with gene activation and is therefore considered a permissive modification. H3K4me3 has been found to be associated with the promoter and 5′-coding regions of active genes in yeast and higher eukaryotes, indicating the specific function of H3K4me3 in the initiation of gene transcription [61, 68]. MLL, a mammalian homologue to the *Drosophila* trithorax protein, is one of the best-characterized members of the H3K4 HMT family [69] and was isolated as a common target of chromosomal translocations observed in human acute leukemia [70]. MLL forms a HMT complex, in conjunction with other core components, including WD40-repeat protein WDR5, RbBP5, and Ash2L [71, 72] (Fig. 2). A deficiency of each of these subunits impairs MLL methyltransferase enzyme activity [71, 72]. H3K27 trimethylation and H3K9 di- and trimethylation contribute to the maintenance of repressive chromatin structure and correlate with gene silencing [64, 73, 74]. Methyl H3K9 serves as a binding site for the chromodomain-containing protein heterochromatin protein HP1, which is essential for the establishment and maintenance of silent chromatin (heterochromatin) [75, 76]. Methyl H3K27 offers a binding site for a chromodomain-containing protein Polycomb, a core component of PRC1, which together with PRC2 (also known as EED-EZH2 complex), has a pivotal role in silencing the developmentally important *Hox* gene and X-chromosome inactivation [64]. PRC2 possesses several core components, EZH2, SUZ12, and EED. EZH2 has a SET domain and possesses an intrinsic HMT enzyme activity, and methylation of H3K27 by EZH2 subsequently recruits PRC1, which exhibits a ubiquitin E3 ligase activity and influences gene transcription via the ubiquitylation of histone H2A [64].

EPIGENETIC REGULATION OF GENES IN IMMUNE SYSTEM

A lingering conundrum of the immune response is how do certain immune/inflammatory cells "remember" whether they should be actively transcribing specific genes, which would facilitate their participation in a given response. Considering the property of inheritance and stability, the epigenetic mechanisms may be involved in the maintenance of cell memory. It has been documented convincingly that the maintenance of memory Th1 and Th2 cells depends on chromatin modifications, including histone acetylation, methylation, and DNA methylation. The Th2 locus containing *Il5, Rad50, Il13*, and *Il4* genes has been well-characterized to exhibit distinct combinations of individual chromatin modifications that correlate with different gene expression patterns [77]. There are three stages during the development of Th1/Th2 cells, including the initiation of Th2 differentiation, reinforcement of Th2 phenotype, and maintenance of Th2 fate. Multiple key transcription factors and signaling pathways have been revealed to be essential for the first two stages, such as IL-4/IL-4R/STAT6 pathway, NFAT, and GATA3 [78, 79]. Deficiency of these molecules inhibits normal Th2 development in vitro and in vivo [77–79]. However, GATA3, a Th2-specific transcription factors, is not absolutely required for IL-4 production during the maintenance stage of Th2 cells, as conditional depletion of GATA3 in differentiated Th2 cells only results in a minor decrease in IL-4 production without affecting the total number of cells capable of producing IL-4 [80]. This observation strongly suggests that differentiated Th2 cells have acquired "cellular memory" of Th2 cytokine production without the further requirement of polarizing signals, which are essential for the initial establishment of this memory. Indeed, subsequent studies of chromatin modifications in Th2 locus during Th1/Th2 development have revealed that Th2 memory was conferred by permissive histone modifications, while repressive modifications were associated with silencing of Th2 locus in differentiated Th1 cells [77]. These data clearly show that the long-term maintenance of Th1/Th2 or "Th1/Th2 memory" correlates with distinct histone modifications.

In addition to the Th1/Th2 paradigm, the newly discovered effector T cell lineage, Th17, has been linked to epigenetic changes in *Il17* locus [81]. Compared with Th1 or Th2 cells, Th17 cells exhibited an enhanced histone acetylation and H3K4me3 at *Il17* promoter region, which is consistent with the general concept discussed above.

Recent study of LPS tolerance has expanded the understanding of epigenetic regulation and maintenance of gene expression in immune system to macrophages [82]. Restimulation of macrophage with LPS results in an attenuated inflammatory response (LPS-tolerance) but maintains an intact antimicrobial response, which is represented by two kinds of genes—tolerizeable and nontolerizeable, respectively. Tolerizeable genes lose the permissive histone modifications—H4Ac and H3K4me3—at their promoter regions during a secondary challenge with LPS, whereas nontolerizeable genes still are able to mount the permissive histone modifications—H4Ac and H3K4me3—in response to a secondary LPS challenge, which correlates with intact capacity in gene transcription. Thus, short-term cellular memory of tolerized macrophages is at least in part a result of the regulation of the gene expression pattern by epigenetic changes at gene-specific loci [83]. This study suggests that analogs to adaptive immune cells, the cells of the innate immune system, are also capable of mounting cell memory in response to specific external stimuli. Regarding the essential roles of innate immune response in pathological conditions, including infections, cancer, and autoimmune diseases, the epigenetic mechanism-mediated innate immune cell memory has significant clinical relevance. Epigenetic memory in innate immune cells may potentially explain long-term maintenance of some disease conditions, presumably through the effects of aberrant cell phenotype and gene expression patterns, such as immunoparalysis induced by severe sepsis or macrophage polarization (M1/M2) in tumorgenesis [84]. Further investigations are required to characterize molecular mechanism(s) responsible for epigenetic changes and the establishment of cell memory.

A number of laboratories have identified that severe, lifethreatening experimental sepsis initially induces a rapid cytokine storm followed by a profound depletion of immune cells in the lung, spleen, and bone marrow [20, 21, 28–31, 85]. As stated above, this is especially apparent in the depletion of DC in these tissues. In the animals that become long-term survivors, DC are repopulated; however, they appear to possess an impaired ability to function in normal innate and acquired immune events. Our previous work has identified that an important cytokine needed to promote and maintain immune responsiveness, IL-12, was chronically altered in CLP-induced experimental severe sepsis [18, 19]. Our data indicate that splenic DC showed a significant impairment in IL-12 production, an essential cytokine directing Th1 immune response [86], at least 6 weeks after CLP. To test if the epigenetic alterations such as histone modifications play a role in the maintenance of impaired IL-12 expression in DC isolated from septic mice, we performed chromatin immunoprecipitation assay, a classical technique to study histone modifications and the binding of transcription factors on promoter regions. We have observed H3K4me3 and H3K27me2 at the promoter regions of *Il12* subunits, *Il12p35* and *Il12p40*, in normal splenic DC. A decreased ratio between H3K4me3 and H3K27me2 at *Il12* promoters was observed in post-septic splenic DC, which correlated with down-regulation of *Il12* gene expression. Furthermore, the decreased ratio of H3K4me3 and H3K27me2 was a result of altered binding of cognate HMT complexes. For example, post-septic splenic DC possessed decreased binding of core components of the MLL complex, WDR5 and RbBP5, but enhanced binding of core components of the PRC2 complex, EED and SUZ12. Thus, the involvement of histone modifications in the maintenance of an altered *Il12* expression pattern in DC long-term following severe sepsis has been indicated.

CONCLUDING REMARKS

Based on the property of inheritance, stability, and its role in immune system, we propose that the long-term maintenance of DC alterations may be a result of epigenetic regulation of

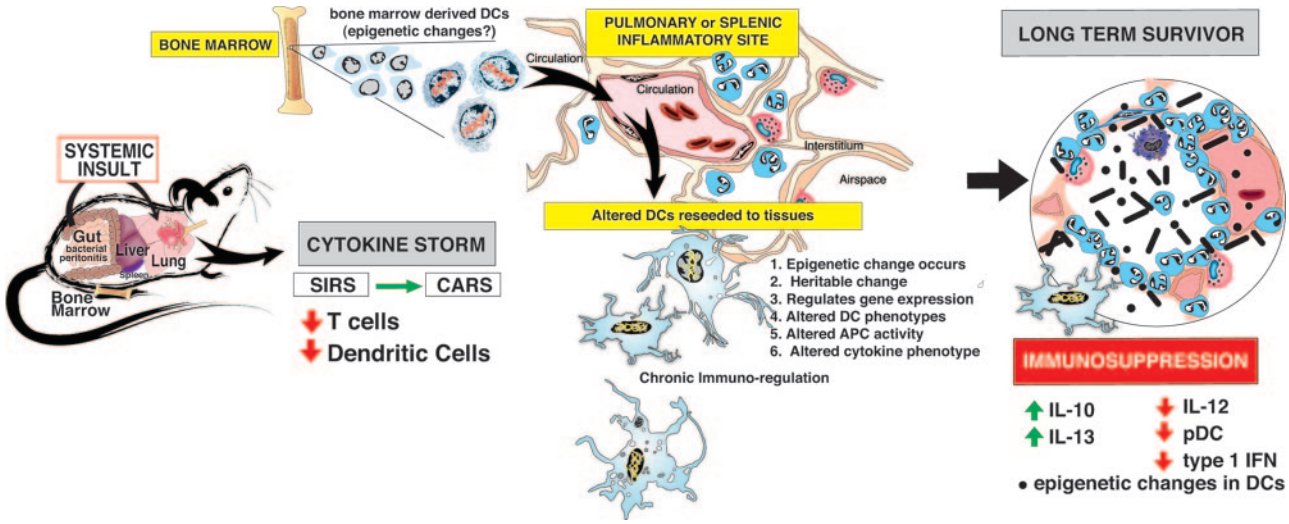

Fig. 3. Schematic of the long-term consequences of severe sepsis on the host's immune status. There is a depletion of immune cells in the distal tissues and reduction of bone marrow cell populations. When the tissues are re-seeded from the bone marrow, profound changes have occurred, some induced by epigenetic alterations, which render the immune cells (DC) impaired to function normally in host responses and immune surveillance. SIRS, Systemic inflammatory response; CARS, compensatory anti-inflammatory response.

expression patterns of key effector molecules. **Figure 3** outlines an evolving hypothesis of the cellular and molecular mechanism by which an initial, overwhelming innate immune response leads to a long-term immunosuppressive state such that the affected host loses the immune capacity to mount an appropriate defense response against a secondary pathological challenge. The key part of this hypothesis includes long-term, abnormal DC differentiation from precursor cells and the maintenance of DC dysfunction mediated by an epigenetic mechanism, which can profoundly control the transcription of specific genes. Then, an important question follows: What are the key molecules/signals mediating epigenetic alterations in DC postepsis? The informative data came from experiments studying LPS-stimulated macrophages [82]. LPS-TLR4 signaling induces a short-term LPS unresponsiveness of macrophages, which is mediated by alterations in histone modifications. This study indicates that TLR signaling is a potential contributor of epigenetic changes; however, signaling through TLR4 alone is not sufficient to build a long-term immune cell memory. In memory T cells, the candidate pathways leading to epigenetic alterations include TCR and cytokine/cytokine receptor signaling pathways. Based on the fact that overwhelmed pathological infection and dysregulated cytokine storm are hallmarks of severe sepsis, it is reasonable to hypothesize that TLR signaling and cytokine/cytokine receptor signaling pathways are potential contributors to the epigenetic changes. In addition, it is highly possible that the synergy between individual TLR signaling exists, resulting in the eventual downstream epigenetic changes, as synergy between different TLR signaling has been shown to promote Th1-polarizing function of DC [87].

Our recent data suggest that long-term alterations, which modify the balance of crucial cell-derived mediators and disrupt the normal functions of these cells, are directed by changes in DC phenotype and alterations in the expression of important host defense genes. Some of these changes are correlated with covalent modification of histone proteins, which can profoundly control the transcription of specific genes. More evidence of the role of the epigenetic alterations in long-term DC dysfunction following acute inflammatory response and further investigation of upstream signaling pathways leading to these epigenetic changes will provide mechanistic insight into the longer-term sequela of sepsis and begin to lay the groundwork for the development of potentially novel cell and mediator-based therapeutic interventions.

ACKNOWLEDGMENTS

This work was supported by grants from the National Institutes of Health received by S. L. K. (HL31237, HL74024, and HL31963). We thank Robin Kunkel for her artistic assistance. We also thank Holly Evanoff and Pam Lincoln for their technical assistance.

REFERENCES

1. Steinman, R. M., Cohn, Z. A. (1973) Identification of a novel cell type in peripheral lymphoid organs of mice. I. Morphology, quantitation, tissue distribution. *J. Exp. Med.* **137,** 1142–1162.
2. Steinman, R. M., Cohn, Z. A. (1974) Identification of a novel cell type in peripheral lymphoid organs of mice. II. Functional properties in vitro. *J. Exp. Med.* **139,** 380–397.
3. Steinman, R. M., Adams, J. C., Cohn, Z. A. (1975) Identification of a novel cell type in peripheral lymphoid organs of mice. IV. Identification and distribution in mouse spleen. *J. Exp. Med.* **141,** 804–820.
4. Banchereau, J., Steinman, R. M. (1998) Dendritic cells and the control of immunity. *Nature* **392,** 245–252.
5. Trombetta, E. S., Mellman, I. (2005) Cell biology of antigen processing in vitro and in vivo. *Annu. Rev. Immunol.* **23,** 975–1028.
6. Banchereau, J., Briere, F., Caux, C., Davoust, J., Lebecque, S., Liu, Y. J., Pulendran, B., Palucka, K. (2000) Immunobiology of dendritic cells. *Annu. Rev. Immunol.* **18,** 767–811.
7. Grosjean, I., Caux, C., Bella, C., Berger, I., Wild, F., Banchereau, J., Kaiserlian, D. (1997) Measles virus infects human dendritic cells and

blocks their allostimulatory properties for CD4+ T cells. *J. Exp. Med.* **186,** 801–812.
8. Wilson, N. S., Behrens, G. M., Lundie, R. J., Smith, C. M., Waithman, J., Young, L., Forehan, S. P., Mount, A., Steptoe, R. J., Shortman, K. D., de Koning-Ward, T. F., Belz, G. T., Carbone, F. R., Crabb, B. S., Heath, W. R., Volladangos, J. A. (2006) Systemic activation of dendritic cells by Toll-like receptor ligands or malaria infection impairs cross-presentation and antiviral immunity. *Nat. Immunol.* **7,** 165–172.
9. Chaux, P., Moutet, M., Faivre, J., Martin, F., Martin, M. (1996) Inflammatory cells infiltrating human colorectal carcinomas express HLA class II but not B7–1 and B7–2 costimulatory molecules of the T-cell activation. *Lab. Invest.* **74,** 975–983.
10. Ghiringhelli, F., Puig, P. E., Roux, S., Parcellier, A., Schmitt, E., Solary, E., Kroemer, G., Martin, F., Chauffert, B., Zitvogel, L. (2005) Tumor cells convert immature myeloid dendritic cells into TGF-β-secreting cells inducing CD4+CD25+ regulatory T cell proliferation. *J. Exp. Med.* **202,** 919–929.
11. Aspord, C., Pedroza-Gonzalez, A., Gallegos, M., Tindle, S., Burton, E. C., Su, D., Marches, F., Banchereau, J., Palucka, A. K. (2007) Breast cancer instructs dendritic cells to prime interleukin 13-secreting CD4+ T cells that facilitate tumor development. *J. Exp. Med.* **204,** 1037–1047.
12. Reddy, R. C., Chen, G. H., Tekchandani, P. K., Standiford, T. J. (2001) Sepsis-induced immunosuppression: from bad to worse. *Immunol. Res.* **24,** 273–287.
13. Angele, M. K., Faist, E. (2002) Clinical review: immunodepression in the surgical patient and increased susceptibility to infection. *Crit. Care* **6,** 298–305.
14. Maier, S., Traeger, T., Entleutner, M., Westerholt, A., Kleist, B., Huser, N., Holzmann, B., Stier, A., Pfeffer, K., Heidecke, C. D. (2004) Cecal ligation and puncture versus colon ascendens stent peritonitis: two distinct animal models for polymicrobial sepsis. *Shock* **21,** 505–511.
15. Hubbard, W. J., Choudhry, M., Schwacha, M. G., Kerby, J. D., Rue III, L. W., Bland, K. I., Chaudry, I. H. (2005) Cecal ligation and puncture. *Shock* **24** (Suppl. 1), 52–57.
16. Walley, K. R., Lukacs, N. W., Standiford, T. J., Strieter, R. M., Kunkel, S. L. (1996) Balance of inflammatory cytokines related to severity and mortality of murine sepsis. *Infect. Immun.* **64,** 4733–4738.
17. Benjamim, C. F., Hogaboam, C. M., Lukacs, N. W., Kunkel, S. L. (2003) Septic mice are susceptible to pulmonary aspergillosis. *Am. J. Pathol.* **163,** 2605–2617.
18. Benjamim, C. F., Lundy, S. K., Lukacs, N. W., Hogaboam, C. M., Kunkel, S. L. (2005) Reversal of long-term sepsis-induced immunosuppression by dendritic cells. *Blood* **105,** 3588–3595.
19. Wen, H., Hogaboam, C. M., Gauldie, J., Kunkel, S. L. (2006) Severe sepsis exacerbates cell-mediated immunity in the lung due to an altered dendritic cell cytokine profile. *Am. J. Pathol.* **168,** 1940–1950.
20. Hotchkiss, R. S., Nicholson, D. W. (2006) Apoptosis and caspases regulate death and inflammation in sepsis. *Nat. Rev. Immunol.* **6,** 813–822.
21. Flohe, S. B., Agrawal, H., Schmitz, D., Gertz, M., Flohe, S., Schade, F. U. (2006) Dendritic cells during polymicrobial sepsis rapidly mature but fail to initiate a protective Th1-type immune response. *J. Leukoc. Biol.* **79,** 473–481.
22. Reik, W. (2007) Stability and flexibility of epigenetic gene regulation in mammalian development. *Nature* **447,** 425–432.
23. Jones, P. A., Baylin, S. B. (2007) The epigenomics of cancer. *Cell* **128,** 683–692.
24. Ballestar, E., Esteller, M., Richardson, B. C. (2006) The epigenetic face of systemic lupus erythematosus. *J. Immunol.* **176,** 7143–7147.
25. Kouzarides, T. (2007) Chromatin modifications and their function. *Cell* **128,** 693–705.
26. Martin, C., Zhang, Y. (2007) Mechanisms of epigenetic inheritance. *Curr. Opin. Cell Biol.* **19,** 266–272.
27. Hotchkiss, R. S., Tinsley, K. W., Swanson, P. E., Grayson, M. H., Osborne, D. F., Wagner, T. H., Cobb, J. P., Coopersmith, C., Karl, I. E. (2002) Depletion of dendritic cells, but not macrophages, in patients with sepsis. *J. Immunol.* **168,** 2493–2500.
28. Hiramatsu, M., Hotchkiss, R. S., Karl, I. E., Buchman, T. G. (1997) Cecal ligation and puncture (CLP) induces apoptosis in thymus, spleen, lung, and gut by an endotoxin and TNF-independent pathway. *Shock* **7,** 247–253.
29. Hotchkiss, R. S., Swanson, P. E., Cobb, J. P., Jacobson, A., Buchman, T. G., Karl, I. E. (1997) Apoptosis in lymphoid and parenchymal cells during sepsis: findings in normal and T- and B-cell-deficient mice. *Crit. Care Med.* **25,** 1298–1307.
30. Efron, P. A., Martins, A., Minnich, D., Tinsley, K., Ungaro, R., Bahjat, F. R., Hotchkiss, R., Clare-Salzler, M., Moldawer, L. L. (2004) Characterization of the systemic loss of dendritic cells in murine lymph nodes during polymicrobial sepsis. *J. Immunol.* **173,** 3035–3043.
31. Ding, Y., Chung, C. S., Newton, S., Chen, Y., Carlton, S., Albina, J. E., Ayala, A. (2004) Polymicrobial sepsis induces divergent effects on splenic and peritoneal dendritic cell function in mice. *Shock* **22,** 137–144.
32. Serbina, N. V., Pamer, E. G. (2006) Monocyte emigration from bone marrow during bacterial infection requires signals mediated by chemokine receptor CCR2. *Nat. Immunol.* **7,** 311–317.
33. Walkley, C. R., Olsen, G. H., Dworkin, S., Fabb, S. A., Swann, J., McArthur, G. A., Westmoreland, S. V., Chambon, P., Scadden, D. T., Purton, L. E. (2007) A microenvironment-induced myeloproliferative syndrome caused by retinoic acid receptor γ deficiency. *Cell* **129,** 1097–1110.
34. Walkley, C. R., Shea, J. M., Sims, N. A., Purton, L. E., Orkin, S. H. (2007) Rb regulates interactions between hematopoietic stem cells and their bone marrow microenvironment. *Cell* **129,** 1081–1095.
35. Quartin, A. A., Schein, R. M., Kett, D. H., Peduzzi, P. N. (1997) Magnitude and duration of the effect of sepsis on survival. Department of Veterans Affairs Systemic Sepsis Cooperative Studies Group. *JAMA* **277,** 1058–1063.
36. Griffith, T. S., Yu, X., Herndon, J. M., Green, D. R., Ferguson, T. A. (1996) CD95-induced apoptosis of lymphocytes in an immune privileged site induces immunological tolerance. *Immunity* **5,** 7–16.
37. Voll, R. E., Herrmann, M., Roth, E. A., Stach, C., Kalden, J. R., Girkontaite, I. (1997) Immunosuppressive effects of apoptotic cells. *Nature* **390,** 350–351.
38. Albert, M. L. (2004) Death-defying immunity: do apoptotic cells influence antigen processing and presentation? *Nat. Rev. Immunol.* **4,** 223–231.
39. Fadok, V. A., Bratton, D. L., Konowal, A., Freed, P. W., Westcott, J. Y., Henson, P. M. (1998) Macrophages that have ingested apoptotic cells in vitro inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving TGF-β, PGE2, and PAF. *J. Clin. Invest.* **101,** 890–898.
40. Kim, S., Elkon, K. B., Ma, X. (2004) Transcriptional suppression of interleukin-12 gene expression following phagocytosis of apoptotic cells. *Immunity* **21,** 643–653.
41. Benjamim, C. F., Hogaboam, C. M., Kunkel, S. L. (2004) The chronic consequences of severe sepsis. *J. Leukoc. Biol.* **75,** 408–412.
42. Abraham, E., Nick, J. A., Azam, T., Kim, S. H., Mira, J. P., Svetkauskaite, D., He, Q., Zamora, M., Murphy, J., Park, J. S., Overdier, K., Dinarello, C. A. (2006) Peripheral blood neutrophil activation patterns are associated with pulmonary inflammatory responses to lipopolysaccharide in humans. *J. Immunol.* **176,** 7753–7760.
43. Ramirez-Carrozzi, V. R., Nazarian, A. A., Li, C. C., Gore, S. L., Sridharan, R., Imbalzano, A. N., Smale, S. T. (2006) Selective and antagonistic functions of SWI/SNF and Mi-2β nucleosome remodeling complexes during an inflammatory response. *Genes Dev.* **20,** 282–296.
44. Garcia-Ramallo, E., Marques, T., Prats, N., Beleta, J., Kunkel, S. L., Godessart, N. (2002) Resident cell chemokine expression serves as the major mechanism for leukocyte recruitment during local inflammation. *J. Immunol.* **169,** 6467–6473.
45. Cohen, J. (2002) The immunopathogenesis of sepsis. *Nature* **420,** 885–891.
46. Venet, F., Tissot, S., Debard, A. L., Faudot, C., Crampe, C., Pachot, A., Ayala, A., Monneret, G. (2007) Decreased monocyte human leukocyte antigen-DR expression after severe burn injury: correlation with severity and secondary septic shock. *Crit. Care Med.* **35,** 1910–1917.
47. Perl, T. M., Dvorak, L., Hwang, T., Wenzel, R. P. (1995) Long-term survival and function after suspected gram-negative sepsis. *JAMA* **274,** 338–345.
48. Martin, G. S., Mannino, D. M., Eaton, S., Moss, M. (2003) The epidemiology of sepsis in the United States from 1979 through 2000. *N. Engl. J. Med.* **348,** 1546–1554.
49. Ertel, W., Kremer, J. P., Kenney, J., Steckholzer, U., Jarrar, D., Trentz, O., Schildberg, F. W. (1995) Downregulation of proinflammatory cytokine release in whole blood from septic patients. *Blood* **85,** 1341–1347.
50. Docke, W. D., Randow, F., Syrbe, U., Krausch, D., Asadullah, K., Reinke, P., Volk, H. D., Kox, W. (1997) Monocyte deactivation in septic patients: restoration by IFN-γ treatment. *Nat. Med.* **3,** 678–681.
51. Oberholzer, A., Oberholzer, C., Moldawer, L. L. (2001) Sepsis syndromes: understanding the role of innate and acquired immunity. *Shock* **16,** 83–96.
52. Nagano, H., Tilney, N. L. (1997) Chronic allograft failure: the clinical problem. *Am. J. Med. Sci.* **313,** 305–309.
53. Lemanske Jr., R. F. (2002) The childhood origins of asthma (COAST) study. *Pediatr. Allergy Immunol.* **13** (Suppl. 15), 38–43.
54. Openshaw, P. J., Dean, G. S., Culley, F. J. (2003) Links between respiratory syncytial virus bronchiolitis and childhood asthma: clinical and research approaches. *Pediatr. Infect. Dis. J.* **22,** S58–S64.

55. Rodgers, G. L., Mortensen, J., Fisher, M. C., Lo, A., Cresswell, A., Long, S. S. (2000) Predictors of infectious complications after burn injuries in children. *Pediatr. Infect. Dis. J.* **19,** 990–995.
56. Kobayashi, M., Takahashi, H., Sanford, A. P., Herndon, D. N., Pollard, R. B., Suzuki, F. (2002) An increase in the susceptibility of burned patients to infectious complications due to impaired production of macrophage inflammatory protein 1 α. *J. Immunol.* **169,** 4460–4466.
57. Berger, S. L. (2007) The complex language of chromatin regulation during transcription. *Nature* **447,** 407–412.
58. Feinberg, A. P. (2007) Phenotypic plasticity and the epigenetics of human disease. *Nature* **447,** 433–440.
59. Kornberg, R. D., Lorch, Y. (1999) Twenty-five years of the nucleosome, fundamental particle of the eukaryote chromosome. *Cell* **98,** 285–294.
60. Olins, D. E., Olins, A. L. (2003) Chromatin history: our view from the bridge. *Nat. Rev. Mol. Cell Biol.* **4,** 809–814.
61. Margueron, R., Trojer, P., Reinberg, D. (2005) The key to development: interpreting the histone code? *Curr. Opin. Genet. Dev.* **15,** 163–176.
62. Zhang, K., Dent, S. Y. (2005) Histone modifying enzymes and cancer: going beyond histones. *J. Cell. Biochem.* **96,** 1137–1148.
63. Rea, S., Eisenhaber, F., O'Carroll, D., Strahl, B. D., Sun, Z. W., Schmid, M., Opravil, S., Mechtler, K., Ponting, C. P., Allis, C. D., Jenuwein, T. (2000) Regulation of chromatin structure by site-specific histone H3 methyltransferases. *Nature* **406,** 593–599.
64. Martin, C., Zhang, Y. (2005) The diverse functions of histone lysine methylation. *Nat. Rev. Mol. Cell Biol.* **6,** 838–849.
65. Tschiersch, B., Hofmann, A., Krauss, V., Dorn, R., Korge, G., Reuter, G. (1994) The protein encoded by the Drosophila position-effect variegation suppressor gene Su(var)3-9 combines domains of antagonistic regulators of homeotic gene complexes. *EMBO J.* **13,** 3822–3831.
66. Jones, R. S., Gelbart, W. M. (1993) The Drosophila Polycomb-group gene enhancer of zeste contains a region with sequence similarity to trithorax. *Mol. Cell. Biol.* **13,** 6357–6366.
67. Stassen, M. J., Bailey, D., Nelson, S., Chinwalla, V., Harte, P. J. (1995) The Drosophila trithorax proteins contain a novel variant of the nuclear receptor type DNA binding domain and an ancient conserved motif found in other chromosomal proteins. *Mech. Dev.* **52,** 209–223.
68. Santos-Rosa, H., Schneider, R., Bannister, A. J., Sherriff, J., Bernstein, B. E., Emre, N. C., Schreiber, S. L., Mellor, J., Kouzarides, T. (2002) Active genes are tri-methylated at K4 of histone H3. *Nature* **419,** 407–411.
69. Milne, T. A., Briggs, S. D., Brock, H. W., Martin, M. E., Gibbs, D., Allis, C. D., Hess, J. L. (2002) MLL targets SET domain methyltransferase activity to Hox gene promoters. *Mol. Cell* **10,** 1107–1117.
70. Gu, Y., Nakamura, T., Alder, H., Prasad, R., Canaani, O., Cimino, G., Croce, C. M., Canaani, E. (1992) The t(4;11) chromosome translocation of human acute leukemias fuses the ALL-1 gene, related to Drosophila trithorax, to the AF-4 gene. *Cell* **71,** 701–708.
71. Wysocka, J., Swigut, T., Milne, T. A., Dou, Y., Zhang, X., Burlingame, A. L., Roeder, R. G., Brivanlou, A. H., Allis, C. D. (2005) WDR5 associates with histone H3 methylated at K4 and is essential for H3 K4 methylation and vertebrate development. *Cell* **121,** 859–872.
72. Dou, Y., Milne, T. A., Ruthenburg, A. J., Lee, S., Lee, J. W., Verdine, G. L., Allis, C. D., Roeder, R. G. (2006) Regulation of MLL1 H3K4 methyltransferase activity by its core components. *Nat. Struct. Mol. Biol.* **13,** 713–719.
73. Kouzarides, T. (2002) Histone methylation in transcriptional control. *Curr. Opin. Genet. Dev.* **12,** 198–209.
74. Cao, R., Zhang, Y. (2004) The functions of E(Z)/EZH2-mediated methylation of lysine 27 in histone H3. *Curr. Opin. Genet. Dev.* **14,** 155–164.
75. Lachner, M., O'Carroll, D., Rea, S., Mechtler, K., Jenuwein, T. (2001) Methylation of histone H3 lysine 9 creates a binding site for HP1 proteins. *Nature* **410,** 116–120.
76. Bannister, A. J., Zegerman, P., Partridge, J. F., Miska, E. A., Thomas, J. O., Allshire, R. C., Kouzarides, T. (2001) Selective recognition of methylated lysine 9 on histone H3 by the HP1 chromo domain. *Nature* **410,** 120–124.
77. Ansel, K. M., Djuretic, I., Tanasa, B., Rao, A. (2006) Regulation of Th2 differentiation and IL4 locus accessibility. *Annu. Rev. Immunol.* **24,** 607–656.
78. Murphy, K. M., Reiner, S. L. (2002) The lineage decisions of helper T cells. *Nat. Rev. Immunol.* **2,** 933–944.
79. Mowen, K. A., Glimcher, L. H. (2004) Signaling pathways in Th2 development. *Immunol. Rev.* **202,** 203–222.
80. Zhu, J., Min, B., Hu-Li, J., Watson, C. J., Grinberg, A., Wang, Q., Killeen, N., Urban Jr., J. F., Guo, L., Paul, W. E. (2004) Conditional deletion of Gata3 shows its essential function in T(H)1-T(H)2 responses. *Nat. Immunol.* **5,** 1157–1165.
81. Akimzhanov, A. M., Yang, X. O., Dong, C. (2007) Chromatin remodeling of interleukin-17 (IL-17)-IL-17F cytokine gene locus during inflammatory helper T cell differentiation. *J. Biol. Chem.* **282,** 5969–5972.
82. Foster, S. L., Hargreaves, D. C., Medzhitov, R. (2007) Gene-specific control of inflammation by TLR-induced chromatin modifications. *Nature* **447,** 972–978.
83. Gantner, B. N., Singh, H. (2007) Immunology: short-term memory. *Nature* **447,** 916–917.
84. Sica, A., Bronte, V. (2007) Altered macrophage differentiation and immune dysfunction in tumor development. *J. Clin. Invest.* **117,** 1155–1166.
85. Hotchkiss, R. S., Swanson, P. E., Freeman, B. D., Tinsley, K. W., Cobb, J. P., Matuschak, G. M., Buchman, T. G., Karl, I. E. (1999) Apoptotic cell death in patients with sepsis, shock, and multiple organ dysfunction. *Crit. Care Med.* **27,** 1230–1251.
86. Hsieh, C. S., Macatonia, S. E., Tripp, C. S., Wolf, S. F., O'Garra, A., Murphy, K. M. (1993) Development of TH1 CD4+ T cells through IL-12 produced by Listeria-induced macrophages. *Science* **260,** 547–549.
87. Napolitani, G., Rinaldi, A., Bertoni, F., Sallusto, F., Lanzavecchia, A. (2005) Selected Toll-like receptor agonist combinations synergistically trigger a T helper type 1-polarizing program in dendritic cells. *Nat. Immunol.* **6,** 769–776.