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Signals from CD28 Induce Stable Epigenetic Modification of the IL-2 Promoter

Rajan M. Thomas,* Ling Gao,* and Andrew D. Wells2*†

CD28 costimulation controls multiple aspects of T cell function, including the expression of proinflammatory cytokine genes. One of these genes encodes IL-2, a growth factor that influences T cell proliferation, survival, and differentiation. Antigenic signaling in the absence of CD28 costimulation leads to anergy, a mechanism of tolerance that renders CD4+ T cells unable to produce IL-2. The molecular mechanisms by which CD28 costimulatory signals induce gene expression are not fully understood. In eukaryotic cells, the expression of many genes is influenced by their physical structure at the level of DNA methylation and local chromatin remodeling. To address whether these epigenetic mechanisms are operative during CD28-dependent gene expression in CD4+ T cells, we compared cytosine methylation and chromatin structure at the IL-2 locus in fully activated CD4+ T cells and CD4+ T cells rendered anergic by TCR ligation in the absence of CD28 costimulation. Costimulation through CD28 led to marked, stable histone acetylation and loss of cytosine methylation at the IL-2 promoter/enhancer. This was accompanied by extensive remodeling of the chromatin in this region to a structure highly accessible to DNA binding proteins. Conversely, TCR activation in the absence of CD28 costimulation was not sufficient to promote histone acetylation or cytosine demethylation, and the IL-2 promoter/enhancer in anergic cells remained completely inaccessible. These data suggest that CD28 may function through epigenetic mechanisms to promote CD4+ T cell responses. The Journal of Immunology, 2005, 174: 4639–4646.

Costimulation by CD28 induces a pattern of gene modulation in CD4+ T cells that is quantitatively and qualitatively distinct from TCR signaling alone (1, 2). This leads to the enhanced expression of transcription factors, growth factors, growth factor receptors, metabolic enzymes, antiapoptotic genes, and cytokines that critically influence T cell proliferation, survival, differentiation, anergy avoidance, and effector function (3). Signals from CD28 also activate histone acetyltransferases (4), enzymes that modify nucleosomes and facilitate chromatin remodeling (5), implying that CD28 costimulation may influence gene expression through epigenetic mechanisms. The IL-2 gene is strongly CD28 dependent and contains multiple nucleosomes positioned upstream of the transcriptional start site (6, 7). In many respects, the IL-2 promoter/enhancer resembles an enhancosome, a regulatory region that is controlled at an epigenetic level through histone acetylation and chromatin remodeling (8–10). DNA methylation and chromatin remodeling have been implicated in the epigenetic control of IL-4 and IFN-γ loci during Th polarization (11), and methylation of CpG dinucleotides upstream of the IL-2 gene has recently been shown to negatively regulate promoter activity in a transient reporter system (12).

To test whether CD28 costimulation induces epigenetic modification of the IL-2 gene, we used bisulfite conversion sequence mapping, chromatin immunoprecipitation (ChIP),3 and nucleosome hypersensitivity analysis to examine DNA methylation and chromatin structure at the IL-2 locus in naive and activated CD4+ T cells. We find that one-third of the CpG dinucleotides in the IL-2 promoter in naive CD4+ T cells are methylated, whereas the local histones are hypoacetylated and incorporated into a chromatin structure that is inaccessible to DNA binding proteins. In response to costimulation through the TCR and CD28, the IL-2 promoter/enhancer is dynamically remodeled to form a fully accessible region over a 2- to 3-day period. This is accompanied by marked acetylation of local histones and significant loss of cytosine methylation at all CpG sites. Importantly, these covalent, epigenetic modifications at the IL-2 gene persist after cessation of mitogenic signaling. Conversely, TCR signaling in the absence of CD28 costimulation, a stimulus that does not support IL-2 gene expression and induces anergy in CD4+ T cells (13), did not result in any of these epigenetic changes at the IL-2 locus. These data suggest that CD28 may influence expression of the IL-2 gene through epigenetic tagging of the promoter/enhancer region.

Materials and Methods

Reagents

Anti-mouse CD3 (145-2C11) and CD28 (37.51) Abs were purchased from BD Pharmingen. 293 cells expressing CTLA-4Ig were obtained from BioExpress. Micrococcal nuclease (MNase) and DNase I were purchased from Roche. Preoptimized primer sets and TaqMan probe for IL-2 mRNA quantification were purchased from Applied Biosystems. PCR primers (see Table I) were obtained from GeneLink. All other reagents were of analytical grade purchased from Sigma-Aldrich.

Mice

C57BL/6 mice, 4–6 wk old, were purchased from The Jackson Laboratory and maintained at the laboratory animal facility of The Children’s Hospital.

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DNA was extracted from proteinase K-digested samples by phenol-chloroform extraction and the pellet was resuspended in a small volume of Tris-EDTA buffer (30 μl). The IL-2 promoter region was PCR amplified in three sections using the primer sets (F1 and R1, F2 and R2, and F3 and R3) shown in the Table I. PCR was performed using iCycler thermal cycler (Bio-Rad) under the following conditions: 5-min initial denaturation at 94°C, followed by 30 cycles of 45 s of denaturation at 94°C, annealing for 1 min at 60°C, and extension for 30 s at 72°C with a final extension step for 10 min at 72°C. The PCR products were separated through a 3% agarose-ethidium bromide gel. Real-time PCR was performed using the same cycling conditions using SYBR Green Supermix (Bio-Rad) and was run on a MyiQ thermal cycler (Bio-Rad).

Nucleosome accessibility analysis of chromatin structure
To determine nucleosome accessibility to chromatin at the IL-2 promoter/enhancer, nuclei were isolated from purified CD4<sup>+</sup> T cells as previously described (14) and resuspended in nuclease digestion buffer. Aliquots of nuclei (1 × 10<sup>6</sup>) were incubated with 50 U of DNase I or MNase for 10 min at 25°C, and reactions were terminated by addition of stop buffer (10 mM Tris-HCl (pH 7.6), 10 mM EDTA, 0.5% SDS, and protease K at 100 μg/ml). DNA was extracted by phenol-chloroform extraction. The IL-2 promoter region was PCR amplified in three sections as in the ChIP procedure. PCR product band intensities were determined using Quantity One software (Bio-Rad), and the amount of PCR product amplified from nuclease-digested samples was normalized to the amount of PCR product amplified from undigested DNA.

Sequence-specific DNA methylation analysis
The extent of CpG DNA methylation at the IL-2 promoter/enhancer was determined by sodium bisulfite conversion, followed by PCR amplification and sequencing of individual clones. DNA (2 μg) from purified CD4<sup>+</sup> T cells was first digested with BamHI and HindIII, then purified by phenol-chloroform extraction. DNA was ethanol-precipitated and washed with 70% ethanol, and the pellet was dissolved in 20 μl of Tris-EDTA buffer. Sodium bisulfite conversion of cytosine to uracil was performed as previously described (15) with some modifications (16). DNA was denatured with 0.3 M NaOH at 37°C for 15 min, then incubated for 16 h at 55°C in a mixture containing 1.72 M sodium metabsulfite, 5.36 M urea, and 0.5 mM hydroquinone. Chemically modified DNA was desalted using the Wizard DNA cleanup system (Promega), desulfonated with 0.3 M NaOH, neutralized with 3 M ammonium acetate, and precipitated with ethanol. Primers (shown in Table I) specific for bisulfite-converted DNA were designed, and the IL-2 promoter/enhancer region was PCR-amplified from the top strand (5'-3') of bisulfite-converted DNA by nested primer PCR. A PCR product of 492 bp derived from converted DNA was gel-purified using the QIAquick gel extraction kit (Qiagen), then cloned into pGEM-T Easy vector (Promega). Plasmid DNA was isolated from 5 ml of overnight cultures using the Wizard Plus miniprep DNA purification system (Promega). Plasmid DNA from individual clones was sequenced using M13 reverse primer. Only sequences derived from fully converted alleles were used for methylation analysis.

Results
CD28 costimulation induces histone acetylation at the IL-2 promoter/enhancer
To study epigenetic modification of the IL-2 gene during T cell activation, we used a well-characterized primary in vitro T and B lymphocyte culture model in which signal 1 is delivered to the T cells by a soluble, agonistic Ab against the TCR/CD3 complex. Accessory B cells in the culture provide FcR-mediated cross-linking of the CD3 Ab as well as physiologic costimulatory signals. CD28 costimulation can also be augmented in this system using agonistic anti-CD28 Ab. CD4<sup>+</sup> T cells from these primary cultures express the IL-2 gene (Fig. 1A), proliferate vigorously (17), and are able to produce IL-2 and IFN-γ in response to restimulation (18, 19). To stimulate T cells in the absence of B7-mediated CD28 costimulatory signals, lymphocytes are cultured with anti-CD3 in the presence of CTLA-4Ig fusion protein. IL-2 gene expression (Fig. 1A) and proliferation (17) by CD4<sup>+</sup> T cells from these cultures are drastically reduced. These cells also exhibit an anergic phenotype, in that they produce less IL-2 and IFN-γ in response to restimulation compared with CD28-costimulated effector cells (18, 19) and fail to proliferate during secondary stimulation (Fig. 1B).
Therefore, IL-2 production and CD4⁺ T cell effector differentiation are dependent upon CD28 costimulation in this system.

Histone acetylation often accompanies gene transcription (20) and is required for the appropriate tissue-specific and context-dependent induction of many genes (9, 21, 22). Nucleosomes are known to be positioned across the IL-2 promoter/enhancer (6, 7), but whether histone acetylation represents an important aspect of nucleosome positioning across the IL-2 promoter/enhancer region in CD4⁺ T cells, we performed ChIP on purified CD4⁺ T cells stimulated in the presence or the absence of CD28 costimulation using an Ab specific for the acetylated form of histone H3 (AcH3). Genomic DNA derived from the same cell extract. Likewise, the lack of detectable AcH3 at the IL-2 locus was not due to a failure of the PCR technique, because the same target regions were amplified efficiently from an aliquot of nonimmunoprecipitated (input) genomic DNA derived from the same cell extract. Moreover, the lack of detectable AcH3 at the IL-2 locus was not due to a failure to enrich AcH3 during the ChIP procedure, because strong histone acetylation was readily detected at the CD3ε promoter (Fig. 2, B and C) in these same cells. Effector CD4⁺ T cells stimulated in the presence of CD28 costimulation showed strong histone acetylation (at least 10-fold over background) within the minimal promoter/enhancer region (regions 1 in Fig. 2A) by 24 h (Fig. 2, B and C). The promoter-distal regions (regions 2 and 3) also showed a lower, but significant, degree of AcH3 (Fig. 2B). Histone acetylation peaked at 48 h and was still apparent by 72 h (Fig. 2, B and C), a time point by which the majority of the proliferative response has ceased in this model (17). Importantly, effector CD4⁺ T cells that had been allowed to rest after the primary response exhibited a significant degree of AcH3 still present at the minimal promoter/enhancer region (Fig. 2, B and C), suggesting that CD28 costimulation results in stable epigenetic tagging of the IL-2 promoter. This may function to facilitate the more rapid transcription of the IL-2 gene observed upon restimulation of effector T cells (12, 23).

In contrast to effector cells, CD4⁺ T cells rendered anergic by activation in the absence of CD28 costimulation exhibited little or no AcH3 across the entire 700-bp region upstream of the IL-2 gene throughout the entire response (Fig. 2, B and C). As with the naive cells, this lack of AcH3 is to some degree specific for the IL-2 locus, because both anergic and effector cells showed a comparable degree of histone acetylation at the CD3ε promoter at each time point (Fig. 2, B and C). These data demonstrate that histone acetylation at the IL-2 promoter/enhancer is dependent upon CD28 costimulation in this system.

CD28 costimulation induces chromatin remodeling at the IL-2 promoter/enhancer

Histone acetylation directly opposes the compaction of nucleosomes into higher order chromatin structures and creates binding sites for chromatin-remodeling enzymes that are capable of repositioning nucleosomes and affecting the accessibility of genomic DNA to transcription factors (5). Because our data from Fig. 2 show that CD28 costimulation can directly promote histone acetylation at the IL-2 promoter/enhancer region in CD4⁺ T cells, we next examined whether CD28 costimulation could likewise promote chromatin remodeling and accessibility of the promoter region to DNA binding proteins. To do this, we used a PCR-based nuclelease hypersensitivity assay, with the same primer sets as those used to probe for the IL-2 promoter in the AcH3 ChIP assay (Fig. 2A). Nuclei were isolated from purified naive, effector, or anergic CD4⁺ T cells and exposed to either MNase, which cuts accessible linker DNA between free nucleosomes, or DNase I, which cuts linker DNA as well as DNA wrapped around free nucleosomes.

In naive CD4⁺ T cells, the genomic DNA spanning the 700-bp region upstream of the transcriptional start site was completely inaccessible to both MNase and DNase I digestion (Fig. 3), indicating that the IL-2 locus in these cells exists in a highly compact and condensed chromatin structure. By 24 h after activation in the presence of CD28 costimulation, the accessibility of the entire region by probing region to MNase had increased by ~50%, whereas DNase I accessibility had increased by nearly 75% (Fig. 3). By 48 h, the 700-bp upstream region was almost completely digested by both enzymes (Fig. 3), suggesting that the chromatin encompassing the entire IL-2 promoter/enhancer in these effector cells is decondensed and wrapped loosely around free nucleosomes. The most
promoter-proximal genomic regions (regions 1 and 2) remained accessible to DNA binding proteins throughout the entire 3-day response, whereas the most distal region (region 3) had begun to close by this time point (Fig. 3). Interestingly, when these effector T cells were allowed to come to rest for 24 h by interruption of TCR signaling, the entire probed region upstream of the IL-2 gene returned to a completely inaccessible configuration similar to that in naive CD4\(^+\) T cells (Fig. 3). This indicates that the chromatin structure at the IL-2 promoter/enhancer and upstream regions is dynamic and is susceptible to local, kinetic variations induced throughout T cell activation.

Remarkably, CD4\(^+\) T cells stimulated in the absence of CD28 costimulation completely failed to remodel chromatin at the IL-2 locus over the entire 4-day response. In contrast to the effector cells, the 700-bp region upstream of the gene remained almost completely inaccessible to MNase or DNase I digestion throughout the duration of the response (Fig. 3). These results demonstrate that TCR-induced chromatin remodeling at the IL-2 promoter/enhancer requires CD28 costimulation and suggest that epigenetic modification of the histone and chromatin structure may represent a mechanism by which CD28 promotes effective T cell responses.

CD28 costimulation results in demethylation of CpG dinucleotides within the IL-2 promoter/enhancer

In addition to local chromatin structure, another epigenetic modification that can affect gene expression is methylation of genomic DNA at CpG dinucleotides (24). DNA methylation within the IL-2 promoter has been shown to affect transcription of IL-2 promoter/reporter constructs (12); therefore, we next tested whether CD28 might influence the degree or pattern of DNA methylation at the IL-2 locus. We first examined the DNA methylation status of four CpG dinucleotides within the IL-2 promoter/enhancer (Fig. 4A). Individual alleles derived from naïve C57BL/6 CD4\(^+\) T cells displayed significant heterogeneity in the pattern of methylation at each CpG dinucleotide within the IL-2 promoter/enhancer and, in general, were not heavily methylated (Fig. 4A). For instance, ~80% of the alleles analyzed contained one or two methylated CpG sites (MeCpG), and roughly 10% were completely unmethylated (Fig. 4, A and D). In contrast, <5% of the alleles were >50% methylated (Fig. 4, A and D). This heterogeneity in the
degree of DNA methylation among individual alleles is not merely a product of a polyclonal T cell repertoire, because naive CD4+ T cells from monoclonal D011.10/SCID and AND/RAG−/−/H11002 TCR-transgenic mice exhibited a similar degree and pattern of methylation at the IL-2 promoter (Fig. 4D). This degree of hypomethylation at the IL-2 promoter/enhancer is characteristic of cells of the T lymphocyte lineage, because naive CD8+ T cells from P14 TCR-transgenic mice showed this same pattern, whereas in contrast, this same region in DNA derived from liver exhibited almost complete methylation at all four CpG dinucleotides (Fig. 4D). This led to a situation in which the average IL-2 promoter/enhancer was ∼35% methylated. However, the most proximal CpG site at −68 bp, located near the TATA box, was highly methylated (80% at the population level). The more distal CpG sites exhibited a second-order decay pattern of MeCpG, such that each CpG distal to the transcriptional start site showed roughly half the degree of methylation as its more gene-proximal neighbor. This decaying pattern of MeCpG appeared to be specific to promoter regions, because the same pattern was observed at the IFN-γ promoter, whereas a dense island of 10 CpG dinucleotides in the IFN-γ intron I enhancer was almost completely methylated in these same cells (our unpublished data).

Effector CD4+ T cells stimulated for 3 days with anti-CD3 and anti-CD28 showed a significant decrease in the average degree of methylation at each CpG site, such that the average IL-2 promoter/enhancer exhibited a 2-fold reduction in total MeCpG compared with that in naive cells (Fig. 4C). This reduction in MeCpG was the result of a major shift in the distribution of methylated alleles, such that there was a 4-fold increase in the frequency of fully demethylated alleles as CD4+ T cells differentiated from naive to effector, and a complete loss of alleles with three or more MeCpG across the IL-2 promoter/enhancer (Fig. 4D). In contrast, CD4+ T cells stimulated through the TCR in the absence of CD28 costimulation showed no significant change in either the average degree of methylation at each CpG site (Fig. 4B) or the distribution of methylated alleles compared with their naive precursors (Fig. 4D). These data show that productive T cell activation is accompanied by significant demethylation of the IL-2 promoter/enhancer region.
Discussion

The mammalian genome is compacted ∼500-fold into a condensed chromatin structure that acts as a physical barrier to DNA binding proteins (25). This structure is dynamic and can be loosened by various biochemical processes, including acetylation of histones at conserved N-terminal tails (5). Acetyl groups directly oppose the tight packing of nucleosomes into higher order structures and also act as binding sites for the recruitment of ATP-dependent nucleosome remodeling complexes that relax DNA-histone interactions (26). Conversely, methylation of CpG dinucleotides, which occurs at high density at silenced genes, serves to recruit histone deacetylases, histone methyltransferase, and chromatin remodeling complexes that promote a closed chromatin conformation (24).

These epigenetic mechanisms are operative during Th1 vs Th2 polarization (27–33). In naive CD4+ T cells, the IL-4 and IFN-γ loci are heavily methylated and are incorporated into condensed chromatin. Differentiation into Th1 effectors is accompanied by the selective opening and demethylation of the IFN-γ locus, whereas the IL-4/IL-13 locus remains closed and methylated. Conversely, upon differentiation into Th2 effector cells, the IL-4/IL-13 locus is opened and demethylated, whereas the IFN-γ locus remains closed and methylated. Global inhibition of either DNA methylation or histone deacetylation during Th polarization results in the failure of differentiating T cells to silence the appropriate set of cytokine genes (28, 29, 34–36).

A significant portion of the minimal promoter/enhancer of the IL-2 gene is wrapped around a nucleosome in mouse EL4 cells (6) and in human Jurkat cells (7). This nucleosome occludes the TATA box of the IL-2 promoter and opposes the binding of multiple transcription factors in vitro (7). The IL-2 promoter has also been shown to bind architectural high mobility group I(Y) proteins involved in DNA bending, a process required for normal IL-2 gene expression (37, 38). In this way, the IL-2 regulatory region resembles that of the IFN-γ gene, a locus that requires histone acetylation and nucleosome remodeling for its normal pattern of expression (9). Chromatin remodeling at the IL-2 locus has been studied in EL4 thymoma cells (6, 39) and, to a lesser extent, in primary CD4+ T cells (39, 40) during the first 16 h of stimulation. These studies showed rapid development of nuclease sensitivity in response to stimulation with PMA or agonistic Abs, and these changes preceded the onset of IL-2 transcription.

Epigenetic histone modifications have not previously been studied at the endogenous IL-2 promoter in CD4+ T cells, and the dynamics of chromatin remodeling in this region throughout the entire primary response have not been examined. Our studies extend previous results to correlate remodeling with histone acetylation and to examine this relationship over an entire 4-day in vitro response in primary CD4+ T cells. We found that histone acetylation and promoter accessibility peak 2 days into the response, a time at which most of the cells are cycling rapidly. This suggests that cell division may have an active role in remodeling of the IL-2 locus during a productive response. In addition, our studies establish that chromatin remodeling and histone acetylation at the IL-2 gene are dependent upon signals from CD28 in this model, a result

FIGURE 4. Analysis of DNA methylation at the IL-2 promoter/enhancer in naive, effector, and anergic CD4+ T cells. The IL-2 promoter region was amplified from sodium bisulfite-treated genomic DNA. Each row represents an individual cloned and sequenced allele from purified naive (A), anergic (B), and effector (C) CD4+ T cells. ○, unmethylated CpG dinucleotide; ●, methylated CpG dinucleotide. All non-CpG cytosines in the endogenous sequence were converted to thymidines in each treated allele, confirming that full conversion was achieved. Twenty-four to 27 clones were sequenced from each cell population. Pie charts represent the percentage of MeCpG at each site. D, The frequency of individual alleles with 4, 3, 2, 1, or 0 MeCpG from liver or from naive T cells purified from P14, AND/RAG–/–, or SCID/D011 TCR-transgenic mice. Data from the naive, anergic, and effector B6 CD4+ T cells in A–C are also depicted. The global percentage of cytosine methylation at the IL-2 promoter in each cell population is shown in the bottom row. The data from anergic and effector cells are representative of at least two separate experiments.
consistent with the coincidence with proliferation and with the previous finding that short term remodeling at the CD28 response element in the IL-2 promoter requires c-Rel (40). Whether nucleosome acetylation and remodeling are required for the tissue-specific and context-dependent expression of the IL-2 gene has not been tested and will require additional study. A remarkable aspect of our data, however, is that maintenance of established AcH3 at the proximal promoter region did not require continued mitogenic signaling, because rested effector CD4\(^+\) T cells still exhibited significant levels of acetylation after the primary response. This implies that histone acetylation could serve as an epigenetic tag by which this gene remains poised for rapid expression when effector or memory cells re-encounter Ag in the periphery (23).

Cytosine methylation at CpG dinucleotides in genomic DNA promotes stable silencing of transcriptionally inactive genes (24). The pattern of genomic DNA methylation changes throughout embryonic development and tissue differentiation, where it promotes the establishment of tissue-specific patterns of gene expression (41, 42). The IL-2 promoter/enhancer region contains several CpG dinucleotides that could render this locus susceptible to epigenetic regulation by DNA methylation. A previous study concluded that these CpG sites were completely methylated in naive CD4\(^+\) T cells (12). However, our analysis shows that these CpG sites are largely unmethylated in naive T cells, which is consistent with the fact that this gene is not silenced in naive T cells. Conversely, we show that liver cells, which do not express the IL-2 gene, exhibit almost complete methylation of CpG dinucleotides within the promoter/enhancer region. The basis for the discrepancy between these two studies is unclear; however, we found that the CpG at −68, which is located near the TATA box, was methylated in >80% of the total alleles in naive T cells, and 95% of the alleles that exhibited any degree of methylation were methylated at this proximal site. Consistent with previous data (12), populations of activated effector CD4\(^+\) T cells showed a 4-fold increase in the frequency of completely unmethylated alleles, which in our model could be accounted for largely by loss of methylation at the −68 site. Together, these results suggest that DNA methylation at this proximal CpG dinucleotide could act as an epigenetic switch that influences subsequent IL-2 transcription. In fact, two lines of evidence specifically suggest that DNA methylation is important for regulation of the IL-2 gene. First, T cells that lack expression of DNA methyltransferase 1 exhibit reduced global DNA methylation (34) and increased expression of the IL-2 gene upon activation (34, 35). Second, artificial methylation of the minimal IL-2 promoter resulted in reduced reporter gene expression in transient transfection assays, whereas mutation of these CpG dinucleotides to abrogate cytosine methylation resulted in increased reporter gene expression (12). The results of these previous studies suggest that the reduced methylation that we observed at the IL-2 promoter in populations of effector, but not anergic, CD4\(^+\) T cells could influence the kinetics and/or level of expression of this gene in response to restimulation.

CD28 costimulation acts to promote T cell-mediated immunity by augmenting the expression of various genes involved in cell survival, cell cycle progression, and effector function (3). Our data from the IL-2 locus together with the recent finding that CD28 costimulation enhances histone acetylation at the IL-5 promoter in developing Th2 cells (43) suggest that CD28 may influence gene expression in T cells through modulating chromatin structure and DNA methylation. It is likely that CD28 costimulation may function similarly in the epigenetic regulation of other genes involved in T cell function, such as those encoding other cytokines and their receptors or those encoding pro- or antiapoptotic factors, for instance. By promoting the epigenetic tagging of sets of differenti-ation-associated genes, CD28 costimulation during initial antigenic exposure could help to program longer term effector and memory T cell fates during an immune response.

The mechanism by which CD28 costimulation induces these epigenetic modifications is not clear. Signals from this costimulatory receptor could promote chromatin remodeling by augmenting global histone acetyltransferase activity in T cells (4) or by activating transcription factors such as NFAT, CREB, AP-1, and NF-κB, which can recruit the p300/CREB-binding protein histone acetyltransferase to the IL-2 promoter (10, 44, 45). Alternatively, the effect of CD28 costimulation on histone acetylation, chromatin remodeling, and DNA methylation could be indirect, either through effects on growth factor signaling or by promoting chromosome decondensation and loss of CpG methylation during cell cycle progression and DNA replication. These two modes are not mutually exclusive and may operate at different phases of the response. For instance, dynamic targeting of chromatin-modifying enzymes to the IL-2 promoter may be important for early IL-2 production by activated naive T cells, whereas indirect remodeling and cytosine demethylation throughout the proliferative phase may poise the locus for rapid expression by effector T cells upon subsequent exposure to Ag. Finally, the failure of anergic CD4\(^+\) T cells in our model to acylate, remodel, and demethylate the IL-2 promoter/enhancer implies that silencing of IL-2 and other effector cytokine genes during tolerance induction may be reinforced through epigenetic mechanisms.

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Disclosures

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