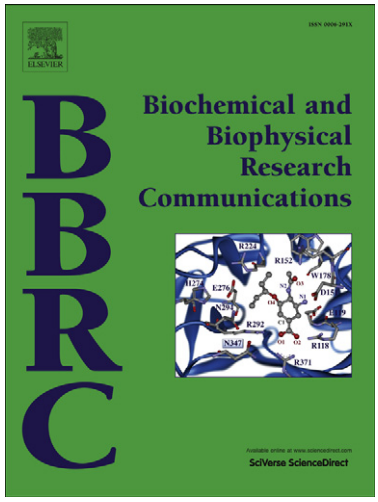

Abnormal DNA methylation of ITGAL (CD11a) in CD4 + T cells from infants with biliary atresia

Rui Dong, Rui Zhao, Shan Zheng, Yijie Zheng, Shudao Xiong, Yiwei Chu

PII: S0006-291X(11)02263-7
DOI: 10.1016/j.bbrc.2011.12.054
Reference: YBBRC 27841

To appear in: *Biochemical and Biophysical Research Communications*

Received Date: 22 November 2011

Please cite this article as: R. Dong, R. Zhao, S. Zheng, Y. Zheng, S. Xiong, Y. Chu, Abnormal DNA methylation of ITGAL (CD11a) in CD4 + T cells from infants with biliary atresia, *Biochemical and Biophysical Research Communications* (2011), doi: 10.1016/j.bbrc.2011.12.054

Abnormal DNA methylation of ITGAL (CD11a) in CD4 + T cells from infants with biliary atresia

Rui Dong[a], Rui Zhao[a], Shan Zheng[a,*], Yijie Zheng[b], Shudao Xiong[b], Yiwei Chu[b]

[a] Department of Pediatric Surgery, Children's Hospital of Fudan University, 399 Wan Yuan Road, Shanghai 201102, China

Rui Dong and Rui Zhao contributed equally to this manuscript and should be considered as co-first authors.

[b] Department of Immunology, Shanghai Medical College, Fudan University, 138 Yixueyuan Road, Shanghai, 200032, China.

[*] Corresponding author. Address: Department of Pediatric Surgery, Children's Hospital of Fudan University, and Key Laboratory of Neonatal Disease, Ministry of Health, 399 Wan Yuan Road, Shanghai 201102, China. Fax: +86 021 64931901; Tel: +86 021 64931007.

E-mail address: szheng@shmu.edu.cn

Acknowledgments: This study received financial support from the National Natural Science Foundation of China (no. 30973139), and The Science Foundation of Shanghai (no. 09JC1402800).

Conflict of interest statement: The authors declare that they have no conflict of interest.

Abstract

Recent evidence indicates that alterations to epigenetic DNA methylation patterns contribute to many autoimmune diseases. Biliary atresia (BA) is a virus-induced autoimmune disease characterized by impaired T cells, which may be due to aberrant DNA methylation. CD11a, a subunit of the β2-integrin LFA-1 (CD11a/CD18) with costimulatory functions, is overexpressed due to hypomethylation of its promoter regulatory elements in CD4+ T cells from patients with many autoimmune diseases. However, it is unknown whether aberrant expression and methylation of CD11a occur in T cells from infants with BA. We aimed to compare the CD11a expression level and the methylation status of the CD11a promoter region in CD4+ T cells from BA infants and healthy controls (HC). We used real-time quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) to examine CD11a mRNA levels in CD4+ T cells from BA and HC infants. Bisulfite sequencing was used to determine the methylation status of the CD11a promoter and flanking regions in CD4+ T cells from BA and HC infants, and in CD4+ T cells with DNA methylation inhibitors. We found that CD11a expression is significantly decreased in BA CD4+ T cells (P=0.007). This was associated with hypermethylation of the CD11a promoter region in CD4+ T cells from infants with BA. Treatment with a DNA methylation inhibitor decreased CD11a promoter methylation and increased CD11a mRNA. Therefore, DNA hypermethylation at the CD11a locus contributes to the lowered expression of CD11a in BA CD4+ T cells.

Keyword: Biliary atresia; DNA methylation; CD11a; T cells

1. Introduction

Biliary atresia (BA) can develop into neonatal cholestasis via undefined mechanisms and is characterized by fibrosclerosing and inflammatory destruction of the extrahepatic and intrahepatic biliary system during the first few weeks of life [1; 2]. Neonatal cholestasis is a devastating disease that leads to cirrhosis, with liver transplantation as the only option for therapy in the majority of cases [3]. The etiology and pathogenesis of bile duct obstruction in children with BA remains largely unknown. One theory suggests that it may result from a primary perinatal hepatobiliary viral infection that elicits autoimmune-mediated bile duct injury [4], primarily associated with CD4+ Th-1 (T helper 1) cell–mediated inflammatory processes [5]. Furthermore, genome-wide association studies have identified BA susceptibility loci on several chromosomes [6; 7]. However, studies carried out in twins demonstrated that non-genetic factors also play an important role in mediating generalized BA pathogenesis [8] even though specific causes of generalized BA remain obscure, as no common (specific) environmental factor(s) that trigger disease progression (either directly or via an autoimmune response) have yet been identified.

DNA methylation is the only genetically programmed DNA modification process in mammals involved in the regulation of several biological processes, including gene transcription, X-chromosome inactivation, genomic imprinting and chromatin modification [9; 10; 11]. DNA methylation plays a critical role in maintaining T-cell function and a growing body of evidence indicates that failure to maintain DNA methylation levels and patterns in mature T cells can result in T-cell-mediated autoimmune responses *in vitro* and autoimmunity *in vivo* [12]. Defective maintenance of DNA methylation may result in the development of

many autoimmune diseases, including systemic lupus erythematosus (SLE), rheumatoid arthritis and multiple sclerosis [13; 14; 15].

Lymphocyte function–associated antigen 1 (LFA-1) (CD11a/CD18, αLβ2) is an integrin that is important in inflammatory and immune responses. LFA-1 is a leukocyte cell surface glycoprotein and promotes essential adhesive interactions by binding members of the intercellular adhesion molecule (ICAM) family [16]. The importance of LFA-1 in inflammatory conditions is evidenced by leukocyte adhesion deficiency syndrome, in which LFA-1 deficiency results in a lack of an inflammatory response and increased susceptibility to infectious diseases [17]. LFA-1 is also important in adhesive interactions between T cells and other cells of the immune system, including macrophages, dendritic cells, and B lymphocytes, and is essential for recruitment into sites of inflammation, antigen-specific T-cell activation, alloreactive responses, cytotoxic T-cell responses, natural killer responses, and B-cell help [18]. ITGAL, the gene encoding CD11a, is located on chromosome 16p11.2, near genes encoding other members of the integrin family, including CD11b and CD11c [19]. Several studies have reported that gene-specific hypomethylation, particularly of the methylation-sensitive autoreactivity-related gene, CD11a (ITGAL), is overexpressed in T cells from patients with SLE and sub-acute cutaneous lupus erythematosus [20; 21; 22; 23]. However, it is unknown whether aberrant expression and methylation of ITGAL occur in T cells from patients with BA.

In this study, we compared CD11a expression in T cells from BA and HC infants. We also compared the methylation status of the CD11a promoter region in these groups. Together, our results provide novel insights into the pathogenesis of BA, and set a foundation for an understanding of the involvement of epigenetic factors in promoting BA.

2. Materials and methods

2.1. Subjects

Twenty-five infants with BA (mean age ± SEM of 58 ± 4.5 days) were recruited from the Outpatient Pediatric Clinic and Inpatient ward at the Children's Hospital, Medical Center of Fudan University. HC (n=20; mean age of 65 ± 6.7 days) were recruited from the Children's Hospital, Medical Centre of Fudan University. BA and HC infants were age- and gender-matched. Pathological diagnosis of BA infants was confirmed independently by two pathologists. Experimental protocols were reviewed and approved by the human ethics committee of the Medical Center of Fudan University and written informed consent was obtained from the parents of all subjects.

2.2. CD4+ T cell isolation and culture

Peripheral blood was collected in heparinized tubes from patients and controls. Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll-Hypaque density gradient centrifugation (Tianjin Haoyang Biological Manufacture Co., Ltd, Tianjin, China) and CD4+ T cells were isolated by positive selection using magnetic beads as described by the manufacturer (Miltenyi Biotec, Bergisch Gladbach, Germany). The purity of enriched CD4+ T cells isolates was evaluated by flow cytometry and was higher than 94%. Where indicated, the PBMCs from BA patients were stimulated with phytohemagglutinin (PHA), and then treated with 1 μM 5-Azacytidine (5-azaC) (Sigma-Aldrich, USA) in RPMI 1640/10% FCS/IL-2 for an additional 72 hours. After that, CD4+ T cells were isolated from treated PBMCs using CD4 beads. This protocol has been shown to be optimal for inducing expression of other methylation-sensitive T cell genes [20].

2.3. RNA isolation and qRT-PCR

Total RNA from CD4+ T cells was isolated using the DNA/RNA Isolation kit (Tiangen Biotech, Beijing, China). Real-time quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) was performed using an ABI 7500 instrument (Applied Biosystems 7500, ABI, Foster City, CA, USA) and mRNA levels were quantified using the QuantiTect SYBR Green RT-PCR kit (TaKaRa Biotech Co., Dalian, China). Serial dilutions of sample RNA were also included to generate a standard curve used to calculate the relative concentrations of transcript in each RNA sample examined. Negative controls (distilled water substituted for RNA) were also run for each sample and β-actin was amplified and used as a loading control. The specific primers used for amplification of CD11a were forward, 5'-CACATCTTTCACACTTCCACCA-3' and reverse, 5'-AGCCTTTAC CCTCACAGTTCACT-3'; and β-actin: forward, 5'-TCCTTCCTGGGC ATGGAGT-3' and reverse, 5'-CAGGAGGAGCAATGATCTTGAT-3'.

2.4. CD11a genomic DNA extraction and bisulfite sequencing

CD11a genomic DNA was isolated from CD4+ T cells using the DNA/RNA Isolation kit (Tiangen). Determination of disulfite conversion was performed using the EpiTect Bisulfite Kit (Qiagen, Germany). Deoxycytosine and deoxymethylcytosine bases were analyzed in a 2.1-kb fragment of the CD11a gene promoter (accession number M87662) and found to contain 29 CG pairs (Table 1). The 5-flanking sequences were identified by bisulfite treatment of purified DNA followed by PCR amplification of a 589-bp fragment located immediately 5' to the CD11a gene transcription start site (-376 to +213, containing 8 CG pairs), 474-bp fragment (-1015 to -541, containing 6 CG pairs), 492-bp fragment (-1486 to -995, containing 9 CG pairs) and 334-bp fragment (-1883 to -1550, containing 6 CG pairs). Fragments were cloned into the pMD19-T vector (TaKaRa Biotech), and 10 independent clones were sequenced for each of the amplified fragments. The following primers were used respectively: fragment (-376 to +213): forward: 5'-AAGGTCCAGAGAAAGCTCTCAC-3'; reverse: 5'-

CTACACCAAACCCTACAATTTCTC -3'; fragment (-1015 to -541): forward: 5'-AAAAAATTGGGTATAGTGGTTT-3'; reverse: 5'- TCTCTTAAAACCAAAAATCAAA -3'; fragment (-1486 to -995): forward: 5'-TGTTATTGGAGAAATGTTTATTTAAA-3'; reverse: 5'- AACCACTATACCCAATTTTTTAAA -3'; fragment (-1883 to -1550): forward: 5'-AAAGTAGTTAGGTGTGGTGGTT-3'; reverse: 5'-CAAAACCACAATAAAATACCCT-3'.

2.5. Statistical analysis

Data are expressed as the mean ± standard error of the mean (SEM). The Student's *t*-test was used to determine statistically significant differences between groups and P-values less than 0.05 were considered significant. All analyses were performed using the SPSS Version 13.0 (SPSS, Chicago, IL) software.

3. Results

3.1. CD11a transcription levels in BA and HC

qRT-PCR was used to detect the mRNA level of CD11a in CD4+ T cells from 25 BA infants and 20 HC. The mean expression levels of CD11a relative to β-actin were significantly lower in BA compared with HC (0.021±0.002 *vs.* 0.031±0.003, P=0.007).

3.2. Regulatory element methylation status of the CD11a promoter in BA and HC

To test whether changes in DNA methylation contribute to CD11a underexpression in BA CD4+ T cells, we detected the methylation status of 29 CG pairs in 2160 bp of the CD11a promoter (position -1920 to +240) containing the Alu elements and transcription binding site (Table 1). Amplified fragments were cloned and 10 clones were sequenced for each amplification product from each subject. Figure 1 shows the average methylation of each of the 29 CG pairs within the region. We found that more than half of the CG pairs appeared unmethylated in the BA and HC infants. When these pairs were removed from the overall combined average, the average methylation status of the 8 CG pairs (positions -1371, -1261, -1253, -1221, -1189, -1157, -1120, and -1110) was found to be significantly higher in BA CD4+ T cells than in HC (Figure 1A and B). In particular, the CG pair at position -1371 was especially hypermethylated.

3.3. Treatment of BA CD4+ T Cells with 5-azaC enhances CD11a mRNA transcription and reduces the mean methylation level

To further confirm the relationship between the average methylation status of the 8 hypermethylated CG pairs within the Alu elements and CD11a mRNA expression, we investigated whether 5-azaC could affect CD11a gene expression in T cells from BA. CD4+ T cells were treated with 1 μM 5-azaC for 72 hours and CD11a mRNA levels were measured by qRT-PCR. The results showed that CD11a increased significantly in the presence of 5-azaC compared with negative controls (0.016±0.002 *vs.* 0.006±0.002, P=0.013). At the same time, we detected the methylation status of 29 CG pairs and found that the methylation level in

CD4+ T cells treated with 5-azaC was significantly decreased compared with untreated controls (Figure 1 C and D), suggesting that methylation levels are critical for the normal regulation of CD11a in CD4+ T cells.

4. Discussion

To our knowledge this study demonstrates for the first time that regulatory elements of CD11a are hypermethylated in CD4+ T cells from infants with BA. We analyzed the methylation status of 29 CG pairs in the promoter region of the CD11a locus known to be associated with CD11a transcription and found them to be hypermethylated in BA CD4+ T cells compared with controls. A deletion analysis showed that a 292-bp (-1401 to -1110) fragment, containing an Alu element [24], upstream of the CD11a gene possesses promoter activity and is essential for CD11a expression. There was no remarkable difference in the methylation status of other fragments of the CD11a gene promoter region compared with healthy controls. The Alu element is important, impacting gene transcription. To our knowledge, Alu elements are a family of short interspersed repetitive elements (SINEs) found exclusively in primates. These elements are approximately 300 base pairs long, are found in excess of one million copies per diploid genome, and are dispersed throughout the human genome. The proliferation of these elements had a significant impact on the architecture of primate genomes. Alu elements are involved in aspects of gene regulation: CpG methylation, gene rearrangement and binding sites of transcription factors. Alu insertions, deletions and recombination have been shown to be involved in many diseases [25]. Hypermethylation in Alu elements could contribute to their general transcriptional inactivity [26] and modulate gene transcription [27; 28]. In the present study, the methylation levels of the Alu elements in the CD11a promoter of BA CD4+ T cells are significantly higher than HCs, especially, the CpG site -1371. The CpG site -1371 is of particular interest, because it is located in the core area of the Alu element [29; 30].

On the other hand, our results also show that CD11a is underexpressed in BA CD4+ T cells compared with HCs, as shown by qRT-PCR analysis. In addition, the DNA methylation inhibitor 5-azaC increases CD11a expression at the mRNA level in CD4+ T cells from infants with BA. Correspondingly, we found that the average methylation levels of the CD11a promoter in the presence of 5-azaC is decreased in CD4+ T cells from infants with BA, especially the CpG site -1371, indicating that it may play a central role in the process of

CD11a gene transcription. The methylation studies indicate that methylation of these sequences can, in fact, modify CD11a promoter function, indicating that the hypermethylation occurring in BA can contribute to low CD11a expression. ITGAL encodes the integrin alpha L chain (CD11a), which is involved in cellular adhesion and costimulatory signaling in the immune system [31]. CD4+ T cells treated with DNA methylation inhibitors overexpress CD11a and become autoreactive, responding to self-class II MHC without the addition of exogenous antigen.

However, this finding is different from the hypomethylation observed in CD4+ T cells from other autoimmune diseases such as SLE and sub-acute cutaneous lupus erythematosus [20; 21; 22; 23]. One reason for this may be that BA is an organ-specific autoimmune disease, not a systemic autoimmune condition. Therefore, it could be that disease-specific methylation defects in CD4+ T cells from infants with BA have unique effects on immune activation, which determine the tissue-specificity of the disorder. In addition, LFA-1 (CD11a) is underexpressed in BA peripheral blood CD4+ T cells, which contrasts with the overexpression observed in the liver and bile duct tissue [32; 33; 34]. It is possible that this correlated with the overexpression of the ligand adhesion molecule ICAM-1, which can bind lymphocyte function–associated antigen LFA-1 (CD11a), resulting in biliary tract destruction and parenchymal damage. Functioning as a double-edged sword, low LFA-1 (CD11a) levels have been shown to enhance the risk of infections, while high LFA-1 (CD11a) levels and high LFA-1 (CD11a) activity have been associated with inflammatory and immune diseases [35]. Both underexpression and overexpression of LFA-1 (CD11a) may play a key role in the pathogenesis of BA and are worthy of further investigation.

In summary, our findings indicate that the hypermethylation of promoter regulatory elements contributes to lower CD11a expression in CD4+ T cells of infants with BA, and that abnormal expression of CD11a may contribute to the pathogenesis of BA. Moreover, these results further emphasize the importance of abnormal DNA methylation in the development of BA disorders and provide insights into the mechanisms that underlie CD11a deficiency, resulting in an inflammatory response and increased susceptibility to virus infections.

References:

[1] J. Hartley, A. Harnden, D. Kelly, Biliary atresia. BMJ 340 (2010) c2383.

[2] J.L. Santos, M. Choquette, J.A. Bezerra, Cholestatic liver disease in children. Curr Gastroenterol Rep 12 (2010) 30-39.

[3] C. Shen, S. Zheng, W. Wang, et al., Relationship between prognosis of biliary atresia and infection of cytomegalovirus. World J Pediatr 4 (2008) 123-126.

[4] C.L. Mack, The pathogenesis of biliary atresia: evidence for a virus-induced autoimmune disease. Semin Liver Dis 27 (2007) 233-242.

[5] C.L. Mack, R.M. Tucker, R.J. Sokol, et al., Biliary atresia is associated with CD4+ Th1 cell-mediated portal tract inflammation. Pediatr Res 56 (2004) 79-87.

[6] M.M. Garcia-Barcelo, M.Y. Yeung, X.P. Miao, et al., Genome-wide association study identifies a susceptibility locus for biliary atresia on 10q24.2. Hum Mol Genet 19 (2010) 2917-2925.

[7] M. Leyva-Vega, J. Gerfen, B.D. Thiel, et al., Genomic alterations in biliary atresia suggest region of potential disease susceptibility in 2q37.3. Am J Med Genet A 152A (2010) 886-895.

[8] B.M. Smith, J.M. Laberge, R. Schreiber, et al., Familial biliary atresia in three siblings including twins. J Pediatr Surg 26 (1991) 1331-1333.

[9] T.M. Geiman, K.D. Robertson, Chromatin remodeling, histone modifications, and DNA methylation-how does it all fit together? J Cell Biochem 87 (2002) 117-125.

[10] W. Reik, J. Walter, Genomic imprinting: parental influence on the genome. Nat Rev

Genet 2 (2001) 21-32.

[11] J. Chow, E. Heard, X inactivation and the complexities of silencing a sex chromosome. Curr Opin Cell Biol 21 (2009) 359-366.

[12] B. Richardson, DNA methylation and autoimmune disease. Clin Immunol 109 (2003) 72-79.

[13] B. Richardson, L. Scheinbart, J. Strahler, et al., Evidence for impaired T cell DNA methylation in systemic lupus erythematosus and rheumatoid arthritis. Arthritis Rheum 33 (1990) 1665-1673.

[14] E. Karouzakis, R.E. Gay, B.A. Michel, et al., DNA hypomethylation in rheumatoid arthritis synovial fibroblasts. Arthritis Rheum 60 (2009) 3613-3622.

[15] S.E. Baranzini, J. Mudge, J.C. van Velkinburgh, et al., Genome, epigenome and RNA sequences of monozygotic twins discordant for multiple sclerosis. Nature 464 (2010) 1351-1356.

[16] S.D. Marlin, T.A. Springer, Purified intercellular adhesion molecule-1 (ICAM-1) is a ligand for lymphocyte function-associated antigen 1 (LFA-1). Cell 51 (1987) 813-819.

[17] H.K. Hawkins, S.C. Heffelfinger, D.C. Anderson, Leukocyte adhesion deficiency: clinical and postmortem observations. Pediatr Pathol 12 (1992) 119-130.

[18] T.K. Kishimoto, R.S. Larson, A.L. Corbi, et al., The leukocyte integrins. Adv Immunol 46 (1989) 149-182.

[19] A.L. Corbi, R.S. Larson, T.K. Kishimoto, et al., Chromosomal location of the genes encoding the leukocyte adhesion receptors LFA-1, Mac-1 and p150,95. Identification of a gene cluster involved in cell adhesion. J Exp Med 167 (1988) 1597-1607.

[20] Q. Lu, M. Kaplan, D. Ray, et al., Demethylation of ITGAL (CD11a) regulatory sequences in systemic lupus erythematosus. Arthritis Rheum 46 (2002) 1282-1291.

[21] Y. Luo, Y. Li, Y. Su, et al., Abnormal DNA methylation in T cells from patients with subacute cutaneous lupus erythematosus. Br J Dermatol 159 (2008) 827-833.

[22] B.C. Richardson, J.R. Strahler, T.S. Pivirotto, et al., Phenotypic and functional similarities between 5-azacytidine-treated T cells and a T cell subset in patients with active systemic lupus erythematosus. Arthritis Rheum 35 (1992) 647-662.

[23] R.L. Yung, B.C. Richardson, Role of T cell DNA methylation in lupus syndromes. Lupus 3 (1994) 487-491.

[24] R.D. Cornwell, K.A. Gollahon, D.D. Hickstein, Description of the leukocyte function-associated antigen 1 (LFA-1 or CD11a) promoter. Proc Natl Acad Sci U S A 90 (1993) 4221-4225.

[25] P. Polak, E. Domany, Alu elements contain many binding sites for transcription factors and may play a role in regulation of developmental processes. BMC Genomics 7 (2006) 133.

[26] S. Kochanek, D. Renz, W. Doerfler, DNA methylation in the Alu sequences of diploid and haploid primary human cells. EMBO J 12 (1993) 1141-1151.

[27] M.A. Trujillo, M. Sakagashira, N.L. Eberhardt, The human growth hormone gene contains a silencer embedded within an Alu repeat in the 3'-flanking region. Mol Endocrinol 20 (2006) 2559-2575.

[28] D.J. Weisenberger, M. Velicescu, J.C. Cheng, et al., Role of the DNA methyltransferase variant DNMT3b3 in DNA methylation. Mol Cancer Res 2 (2004) 62-72.

[29] P.L. Deininger, M.A. Batzer, Alu repeats and human disease. Mol Genet Metab 67 (1999)

183-193.

[30] D.J. Rowold, R.J. Herrera, Alu elements and the human genome. Genetica 108 (2000) 57-72.

[31] M. Tometten, S. Blois, A. Kuhlmei, et al., Nerve growth factor translates stress response and subsequent murine abortion via adhesion molecule-dependent pathways. Biol Reprod 74 (2006) 674-683.

[32] M. Davenport, C. Gonde, R. Redkar, et al., Immunohistochemistry of the liver and biliary tree in extrahepatic biliary atresia. J Pediatr Surg 36 (2001) 1017-1025.

[33] P.W. Dillon, D. Belchis, K. Minnick, et al., Differential expression of the major histocompatibility antigens and ICAM-1 on bile duct epithelial cells in biliary atresia. Tohoku J Exp Med 181 (1997) 33-40.

[34] P. Dillon, D. Belchis, T. Tracy, et al., Increased expression of intercellular adhesion molecules in biliary atresia. Am J Pathol 145 (1994) 263-267.

[35] K. Zen, C.A. Parkos, Leukocyte-epithelial interactions. Curr Opin Cell Biol 15 (2003) 557-564.

Figure legends

Fig. 1 CD11a promoter methylation patterns in CD4+ T cells from BA and HC infants. (A) and (B) There was no difference in the mean methylation status for each of the 29 CG pairs in CD4+ T cells from BA and HC infants (0.15 versus 0.47, P=0.11). The combined average methylation status of 8 CG pairs (positions -1371, -1261, -1253, -1221, -1189, -1157, -1120, and -1110) in BA was higher than in HCs (0.71 versus 0.36, P<0.0001). Methylation at position -1371 was significantly higher in BA compared with HC infants (0.85 versus 0.15, P<0.0001). (C) and (D) There was no difference in the mean methylation status for each of the 29 CG pairs in CD4+ T cells from untreated (control) and 5-azaC treated cells (0.15 versus 0.25, P=0.16). The combined average methylation status of 8 CG pairs (positions -1371, -1261, -1253, -1221, -1189, -1157, -1120, and -1110) in the 5-azaC group was lower than controls (0.64 versus 0.39, P<0.0001). Methylation at position -1371 was significantly lower in the 5-azaC group than controls (0.81versus 0.20, P<0.0001).

Table legends

Table 1: DNA sequence of the CD11a promoter. The nucleotide sequence for the 5' end of the CD11a gene is displayed. Nucleotide +1 corresponds to the first major transcription start site, and the nucleotides preceding it are represented by negative numbers. The Alu sequence is underlined. The 29 CG pairs are boxed.

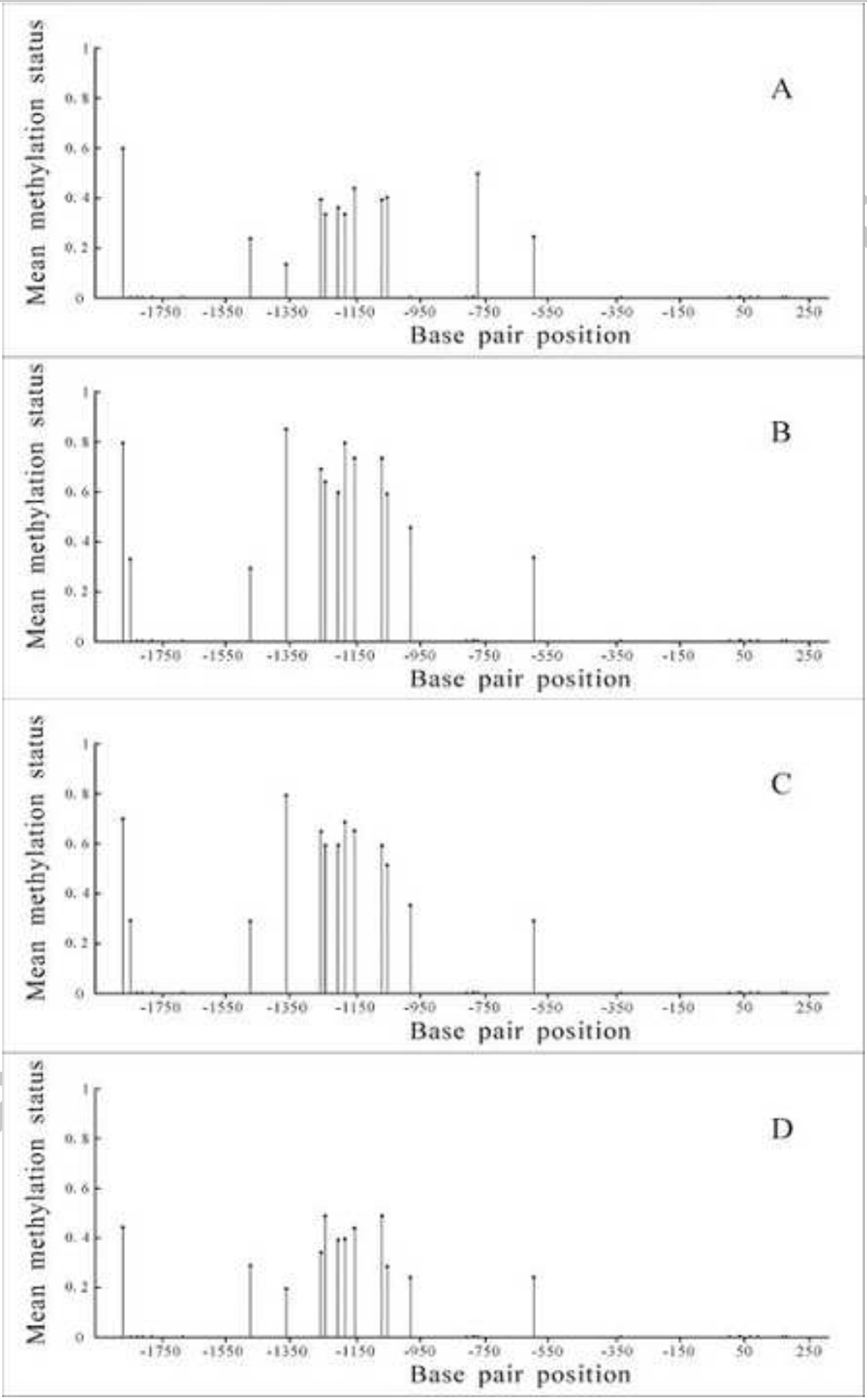

Table 1

-1920 GCAAGGTAGAGAGACCCCCATGTCTACAAAAAATAAAAAAGTAGTCAGGTGTGGTGGCCC
-1860 GCACCTGTAGTTCCAGCTACTCAGGACGCTGAGGTGGGAGAATGACTCGAGCCCGTGAGG
-1800 TTGAGGCTGCAGTGAGCCGAGATCACACCACTCCAGCCTAGGCAAGAGTGAGACCCTCTC
-1740 TCAAAAAAAAAAAAAAAAAAAAAAAAAAAAGAACCATCAAAATGTTTTGCACAGCAGTCA
-1680 CGCCATTTTACATTTCTGCCAGCAATGTGCACCAGGCTTCCAGTTTCTTCACATCTTCAC
-1620 TAACTCTTATTTCCTTTGCTTTAAACTCTAACCATCAAAGTAGGTGTAAAGGGTATCTCA
-1560 CTGTGGTTTGATTTGCATTTCTCTAATGACTAATAGTGTTAAGTATCATTTCATGTGCAT
-1500 GTTGGCCATTTATATGTCATTGGAGAAATGTCTACTCAAACCGTTTGCTCATTTAGAAAC
-1440 TTAGGTAGGTTGGTCTGAGTGCAGTGGTGTTTAAAACTAATTTTTTTTTGAGACAAAGTC
-1380 TCACTCTGTCGCCCAGGCTGGAATGCAATGGTGAGATCTTGGCTCACTACAACCTCCATC
-1320 TCCTGGGTTCAAGCAATTCTTCTGCCTCAGCCTCTCAAGTAGCTGGGATTACAGGCATGC
-1260 GCCACCACGCCTGGCTAATTTTTGTATTTTTGGTAGAGACGGGGGTTTCTCCATGTTGGC
-1200 CAGGCTGGTCTCGAACTCTTGACCTCAGGTGATCCACCTGCCTCGGCCTCCACAGGGCTA
-1140 GGATTAGAGGTGTGAGCCACCGCACCAGGCCGTTTAAAACTAATGGAGCACAACCAGTTA
-1080 CCAATATCTTTGTTCCTTCTCCACTCCCTCTGCTTCAACTTGACTAGCCTAAAATAAATA
-1020 AATTTAAAAAACTGGGCACAGTGGCTCACACCTGTAATCCCAGCACTTTGGGAGGCCGAG
-960 GCAGGAGGATTACTTGAGCATAGGAGTTCAAGATCAGCCTGGGCAACTAGTGAAAAACCA
-900 TCTCAAAAAAGAAAAATTAGCCAGGCATGGTGGCATGCACCTGTGGTTTCAGCTACTTAG
-840 AGCAGAGGTGGAGGATCGCTTGATTCTGGAGTTCAAGGTTGCATTGAGCTGTGATCGCGC
-780 CAGTGCACTCTCGCTTGGGTGACAGAGTAAGACCTTGTCTCAAAAAATTTAAAACAAAAC
-720 AAAAAAAACTGGTTATTTGTCTTTTTATTGGTGAATTATAAGAGTTTTAAAAAATATATT
-660 CTGGAAACAAATCCCTTATTAGAGATATGATTTGCAAATATTTTCTCCAATTTTTTTTTT
-600 TTTTAAAGACAAAGTTTCACTTTGTCGCCCAGGCTGGTCTTGATTCCTGGCTTCAAGAGA
-540 TGCTCTTACCTCCACCTCCTGAAGCCCAAAGGGCTGGAATTACAGCCAGTGAGCCTGCAC
-480 CCAGCCTCCAATTCTTTAGATTTTACATTTTAGAACCAAAATGGGTTAAATACACTGTTC
-420 TGTAATCTGCTCTTTTCTTTAATAGTAGTTCATGTACATCTTTCAAGGTCCAGAGAAAGC
-360 TCTCACTTTCTCCCCGTTTTATTTTTCCTTCCCTCATTCTTTTTCACTGCTGCATAGCAT
-300 TCCATTGTAATTTTGCCACTGTTTATTAGACCAGTCCTCTGCTGAGCTTTACAGAGCCCT
-240 TAGTTGGATGTTAGTGAGAACCATGACAGCAGTGAGACTGTCATCTCCCTGACATGCTGT
-180 CAGCTTTTGGATGATGTGAAAATGCAAGCAGGCACAGGAAATGTCTCTAACTTGCTTACA
-120 CTTCCTCCCTGAACCCTGCGGTTTCACAACTCCTGCAGGCACACCTCCCTCCCCGCCTGC
-60 CAGTGTCACCAGCCTGTTGCCTCTGTGAGAAAGTACCACTGTAAGAGGCCAAAGGGCATG
+1 ATCATTTTCCTCTTTCACCCTGTCTAGGTTGCCAGCAAATCCCACGGGCCTCCTGACGCT
+61 GCCCCTGGGGCCACAGGTCCCTCGAGTGCTGGAAGGATGAAGGATTCCTGCATCACTGTG
+121 ATGGCCATGGCGCTGCTGTCTGGGTTCTTTTTCTTCGGTAGGCAAGGGAGGAGGCAGGGG
+181 AGGGACATGTGTCTGTGACCAGAGAAACTGCAGGGCTTGGTGCAGCTGGAGTAAACAAGG

> CD11a expression is significantly decreased in BA CD4+ T cells.

> CD11a promoter region in BA CD4+ T cells is hypermethylation.

> DNA methylation inhibitor decreased CD11a promoter methylation and increased CD11a mRNA in BA CD4+ T cells.