CD11a polymorphisms regulate T_H2 cell homing and T_H2-related disease

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Background: T_H2-dependent diseases vary in severity according to genotype, but relevant gene polymorphisms remain largely unknown. The integrin CD11a is a critical determinant of allergic responses, and allelic variants of this gene might influence allergic phenotypes.
Objective: We sought to determine major CD11a allelic variants in mice and human subjects and their importance to allergic disease expression.
Methods: We sequenced mouse CD11a alleles from C57BL/6 and BALB/c strains to identify major polymorphisms; human CD11a single nucleotide polymorphisms were compared with allergic disease phenotypes as part of the international HapMap project. Mice on a BALB/c or C57BL/6 background and congenic for the other strain's CD11a allele were created to determine the importance of mouse CD11a polymorphisms *in vivo* and *in vitro*.
Results: Compared with the C57BL/6 allele, the BALB/c CD11a allele contained a nonsynonymous change from asparagine to aspartic acid within the metal ion binding domain. In general, the BALB/c CD11a allele enhanced and the C57BL/6 CD11a allele suppressed T_H2 cell–dependent disease caused by the parasite *Leishmania major* and allergic lung disease caused by the fungus *Aspergillus niger*. Relative to the C57BL/6 CD11a allele, the BALB/c CD11a allele conferred both greater T-cell adhesion to CD54 *in vitro* and enhanced T_H2 cell homing to lungs *in vivo*. We further identified a human CD11a polymorphism that significantly associated with atopic disease and relevant allergic indices.
Conclusions: Polymorphisms in CD11a critically influence T_H2 cell homing and diverse T_H2-dependent immunopathologic states in mice and potentially influence the expression of human allergic disease. (J Allergy Clin Immunol 2014;133:189-97.)

Key words: *Asthma, allergic disease, CD11a, T_H2 cell, homing, polymorphism, allele, congenic, biomarker*

Abbreviations used
AHR: Airway hyperreactivity
CAMP: Childhood Asthma Management Program
LD: Linkage disequilibrium
LFA-1: Leukocyte function–associated antigen 1
MIBD: Metal ion binding domain
OVA: Ovalbumin
PE: Phycoerythrin
Rag: Recombination-activating gene
SNP: Single nucleotide polymorphism
WT: Wild-type

The mechanisms that control the recruitment, or homing, of T_H cells collectively represent an essential immune regulatory checkpoint that crucially influences outcomes of diverse infectious and inflammatory processes.[1] The homing of T_H2 cells is specifically regulated through the expression and regulation of leukocyte function–associated antigen 1 (LFA-1), representing the heterodimeric association of CD18 and CD11a.[2] In the absence of stimulation, LFA-1 is maintained in a low-affinity state that weakly binds the LFA-1 receptor CD54 (intercellular adhesion molecule 1) through the inserted (I) domain of CD11a.[3-7] High concentrations of divalent cations stabilize and chemokines activate LFA-1 to a high-affinity state, allowing circulating T cells to tightly adhere to CD54 on endothelial surfaces and extravasate to sites of inflammation and infection.[5,8]

In part, the importance of LFA-1 for T_H2 cell homing is determined by successive rounds of cell division during which LFA-1 expression is maintained or enhanced while expression of alternate homing integrins is reduced.[9] Blockade or genetic deletion of LFA-1 does not interfere with the induction of robust T_H2 responses but precludes efficient T_H2 cell homing to sites of infection and inflammation without interfering with the development and homing of other T_H effector subsets.[9] Therefore selective blockade of T_H2 cell homing through LFA-1 antagonism results in diverse phenotypes in mice, including reduced expression of allergic lung disease and improved control of the intramacrophage parasite *Leishmania major* in C57BL6 and BALB/c mice.[9] The effect of LFA-1 deletion in BALB/c mice is particularly striking, converting the normally progressive and lethal *L major* infection into a transient illness that more closely resembles the disease course of C57BL/6 mice.[9]

C57BL/6 mice control *L major* infection by mounting a dominant T_H1 immune response, whereas BALB/c mice do not control the infection because of production of an aberrant T_H2-predominant antiparasite response.[10,11] Although several genetic loci have been shown to contain genes that promote resistance to *L major*,[12] the

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specific genetic causes for the dominant T_H2 response and susceptibility in BALB/c mice have yet to be clearly defined.[13]

Airway challenge of C57BL/6 and BALB/c mice with the same allergen also produces variable immune and physiologic responses, the latter assessed as airway hyperreactivity (AHR), although allergic eosinophil- and T_H2-predominant inflammation is consistently observed.[14] The many genetic factors that underlie the mouse allergic lung inflammatory response[15] overlap considerably with those influencing the mouse response to *L major* infection and include CD11a. BALB/c mice that are deficient in CD11a demonstrate impaired T_H2 cell homing to the site of infection and arrested progression of *L major* infection, which is similar to the healer phenotype observed in the C57BL/6 mouse strain.[9] The apparent increase in resistance in the absence of the BALB/c CD11a allele suggests that the BALB/c CD11a allele is an important factor in the pathogenesis of *L major* in the BALB/c mouse strain. As a gene located within a genomic region identified for carrying *L major* resistance in C57BL/6 mice, it is possible that polymorphisms in CD11a might in part determine the divergent immune and physiologic outcomes of BALB/c and C57BL/6 mice in models of *L major* infection and asthma.

To investigate the existence of polymorphisms in CD11a, we sequenced and identified strain-specific polymorphisms in CD11a of BALB/c and C57BL/6 mice and created mice congenic for CD11a on the BALB/c and C57BL/6 backgrounds to test the effect of CD11a alleles in the T_H1- and T_H2-mediated disease models of *L major* infection and allergic lung disease.

METHODS

Mice

Animal studies were conducted under protocols approved by Baylor College of Medicine's Institutional Animal Care and Use Committee; mice were all housed under specific pathogen-free conditions and were fed standard mouse chow *ad libitum*. BALB/c, C57BL/6, and recombination-activating gene (Rag) $1^{-/-}$ mice were purchased from Jackson Laboratories (Bar Harbor, Me). $Rag2^{-/-}$ mice were acquired from Taconic (Hudson, NY). $CD11a^{-/-}$ mice on the BALB/c (10 generations backcrossed) and C57BL/6 (8 generations backcrossed) backgrounds were provided by Dr C. M. Ballantyne.[16] CD11a-congenic mice on the BALB/c ($Balb^{C57\text{-}CD11a}$) and C57BL/6 ($C57^{balb\text{-}CD11a}$) backgrounds were created by crossing $CD11a^{-/-}$ mice of one background with the wild-type (WT) strain of the other background. Progeny were then backcrossed to the parent $CD11a^{-/-}$ strain, selecting for CD11a expression on CD4 T cells for 8 generations. Homozygous congenic or littermate $CD11a^{-/-}$ mice were recreated by crossing eighth-generation heterozygous mice and selecting for complete loss of the targeting construct by using PCR or CD11a by using flow cytometry, respectively.

L major infection

L major (strain MRHO/SU/59/P/LV39) was cultured and mouse infection was established, as previously described.[17]

Macrophage phenotype

Popliteal lymph nodes were harvested from 3-week *L major*–infected mice and processed for total RNA by using Trizol (Invitrogen, Carlsbad, Calif), according to the manufacturer's methods. Primer sets (Applied Biosystems, Foster City, Calif) for inducible nitric oxide synthase (iNOS; Mm0044 0502_m;), Arg1 (Mm00475988_m1), found in inflammatory zone 1 (Fizz1; Mm00445109_m1), and 18s RNA (4319413E-0710034) were used to quantify mRNA with the TaqMan One-Step RT-PCR Master Mix (4309169, Applied Biosystems) using the 7500 Real-Time PCR System (Applied Biosystems). Signals were used to calculate relative expression levels in accordance with standard practice and expressed as the relative expression ratios of select genes.

Dynamic adhesion assay

Adhesion of $CD4^+$ cells was quantitated by means of video microscopy under continuous flow conditions *in vitro*.[18,19] For further information, see the Methods section in this article's Online Repository at www.jacionline.org.

Flow cytometry

Lymphocytes were stained with CD4-Pacific Blue, CD3–allophycocyanin, and CD11a–phycoerythrin (PE), CD11b-PE, or CD11c-PE (all from BD PharMingen, San Jose, Calif) by using standard staining methods.

Adoptive transfer of ovalbumin-specific $CD4^+$ T cells

Mice were challenged with ovalbumin (OVA)–alum weekly for at least 3 weeks, spleens were harvested, and $CD4^+$ cells were collected, selected under T_H2 responses, differentially labeled fluorescently, and injected into strain-specific Rag-deficient mice for comparison of T_H2 cell homing efficiency. For further information, see the Methods section in this article's Online Repository.

Allergic airway disease

Mice were challenged with a clinical isolate of 4×10^5 *Aspergillus niger* conidia every 2 days for 8 challenges and assessed for allergic airway disease, as previously described.[20]

Human single nucleotide polymorphism analysis and correlation

Human studies were conducted under Institutional Review Board–approved protocols at Harvard Medical School and Brigham and Women's Hospital.

Genotyping and data management

Of the 1041 participating parent-child trios in the Childhood Asthma Management Program (CAMP) trial,[21] 92.9% are included in this analysis. Using data from European Americans (CEU) in the International HapMap project,[22] we applied a linkage disequilibrium (LD)–tagging algorithm (minor allele frequency, ≥10%; $r^2 > 0.8$) to capture common variation in CD11a and its 10-kb flanks.[23] For this study, additional single nucleotide polymorphisms (SNPs) were genotyped to evaluate reported functional variation (r_s) within the genomic region of CD11a. All SNPs were genotyped with the SEQUENOM iPLEX platform (Sequenom, San Diego, Calif).[24] The 8 polymorphic SNPs successfully genotyped capture of greater than 84% of the HapMap SNPs with a minor allele frequency of 10% or greater in CD11a and its 10-kb flanks in CEU trios at an r^2 value of greater than 0.8.

Statistical analysis

Mouse statistics were performed, as described previously.[25] For human data, the family-based association analyses in CAMP were performed with the family-based association test statistic implemented in GoldenHelix PBAT version 3.6.[26] In family-based samples Hardy-Weinberg equilibrium was tested in parental data.

RESULTS

Identification of polymorphisms in CD11a

To determine whether CD11a polymorphisms distinguish the divergent responses of BALB/c and C57BL/6 mice to *L major* infection, we first sequenced the full CD11a mRNA from C57BL/6 and BALB/c mice (GenBank no. JN986841.1, Fig 1). Of 3 variances from the canonical C57BL/6 sequence, 2 of the polymorphisms were found in the metal ion binding domain (MIBD) of CD11a, with the C57BL/6 allele encoding asparagine at position 598 and proline at position 603 and the BALB/c allele encoding aspartic acid and