The SIGLEC14 null allele is associated with *Mycobacterium tuberculosis*- and BCG-induced clinical and immunologic outcomes


a Univ. of Washington, Seattle, WA, USA
b Univ. of California San Diego, La Jolla, CA, USA
c South African Tuberculosis Vaccine Initiative, Institute of Infectious Disease and Molecular Medicine, Division of Immunology, Department of Pathology, Univ. of Cape Town, Cape Town, South Africa
d Oxford University Clinical Research Unit, Hospital for Tropical Diseases, Ho Chi Minh City, Viet Nam
e Pham Ngoc Thac Hospital for Tuberculosis and Lung Disease, Ho Chi Minh City, Viet Nam
f Peter Doherty Institute for Infection and Immunity, The University of Melbourne, Australia
g Nuffield Department of Medicine, University of Oxford, UK
h Institute of Biological Chemistry, Academia Sinica, Taipei, Taiwan

ARTICLE INFO

Article history:
Received 10 October 2016
Received in revised form 12 February 2017
Accepted 19 February 2017

Keywords:
SIGLEC
Tuberculosis
Mycobacterium tuberculosis

ABSTRACT

Humans exposed to *Mycobacterium tuberculosis* (Mt bn) have variable susceptibility to tuberculosis (TB) and its outcomes. Siglec-5 and Siglec-14 are members of the sialic-acid binding lectin family that regulate immune responses to pathogens through inhibitory (Siglec-5) and activating (Siglec-14) domains. The *SIGLEC14* coding sequence is deleted in a high proportion of individuals, placing a *SIGLEC5*-like gene under the expression of the *SIGLEC14* promoter (the *SIGLEC14* null allele) and causing expression of a Siglec-5 like protein in monocytes and macrophages. We hypothesized that the *SIGLEC14* null allele was associated with Mt b replication in monocytes, T-cell responses to the BCG vaccine, and clinical susceptibility to TB. The *SIGLEC14* null allele was associated with protection from TB meningitis in Vietnamese adults but not with pediatric TB in South Africa. The null allele was associated with increased IL-2 and IL-17 production following ex-vivo BCG stimulation of blood from 10 week-old South African infants vaccinated with BCG at birth. Mt b replication was increased in THP-1 cells overexpressing either Siglec-5 or Siglec-14 relative to controls. To our knowledge, this is the first study to demonstrate an association between SIGLEC expression and clinical TB, Mt b replication, or BCG-specific T-cell cytokines.

© 2017 Elsevier Ltd. All rights reserved.

1. Introduction

*Mycobacterium tuberculosis* (Mt b) is a leading infectious cause of morbidity and mortality worldwide. In 2014 there were an estimated 9.4 million incident cases of tuberculosis (TB) disease and 1.5 million deaths [1]. There are substantial differences in individual susceptibility to tuberculosis [2–4]. Evidence from twins, Mendelian studies in children, genome wide linkage studies, candidate gene association studies, and genome-wide association studies suggest that human genetic factors mediate susceptibility to TB disease [5–10]. The human innate immune system is critical in the early response to Mt b and the effector mechanisms that kill the bacillus [2,5,11]. Macrophages are a major reservoir of Mt b and the
success of the bacterium depends in part on its ability to circumvent macrophage killing mechanisms [12,13]. The host genetic factors that influence the macrophage response to Mtb are not well understood.

Sialic acid-binding immunoglobulin-like lectins (Siglecs) are a family of cell-surface transmembrane receptors that contain an amino-terminal sialic acid-binding site and are expressed on many immune cells including macrophages [14,15]. All host cells express sialic acids on their surface and recognition of these “Self Associated Molecular Patterns” by Siglecs allows host immune cells to distinguish between self and non-self [16–18]. Consistent with their role in the inhibition of an inappropriate autoimmune inflammatory response, most Siglecs have cytoplasmic domains that contain immunoreceptor tyrosine-based inhibitory motifs (ITIMs) and induce immunosuppressive signaling events through interaction with tyrosine phosphatases such as SHP1 and SHP2. A smaller number of Siglecs associate with an immunoreceptor tyrosine-based activation motif (ITAM) via DAP12 and induce an activation signal through interaction with spleen tyrosine kinase (SYK)[17,19]. Some pathogenic organisms are able to bind host Siglecs. Siglecs mediate capture of HIV by dendritic cells [20] and entry of varicella zoster virus into oligodendroglial cells [21] while certain bacteria are able to induce Siglec signaling in a sialic acid-dependent [22–24] or sialic acid-independent manner [25]. The cell envelope of Mtb contains a variety of glycoconjugates [26], though it is not known whether Mtb is able to bind to Siglecs molecules or induce Siglec signaling.

SIGLEC5 and SIGLEC14 encode two human Siglec molecules that are expressed on host innate immune cells including granulocytes and macrophages; Siglec-5 is also expressed on B-cells at lower levels [27]. The two genes share more than 99% sequence homology in the exons encoding their ligand-binding domains [28] but have opposing intracellular signaling effects due to the presence of an ITIM in the cytoplasmic domain of Siglec-5 and an ITAM in the cytoplasmic domain of the Siglec-14/DAP12 complex [27,29]. The two molecules have been postulated to represent a paired receptor system, in which the inhibitory effect of Siglec-5 is counterbalanced by the activating effect of Siglec-14 [28–30]. Some individuals lack expression of Siglec-14 due to a SIGLEC14 deletion polymorphism between the two genes on chromosome 19. The deletion, termed the SIGLEC14 null allele, eliminates Siglec-14 protein expression but simultaneously places a SIGLEC5-like gene fusion product under the control of the SIGLEC14 promoter [27–29]. Macrophages of null allele homoygotes express only the inhibitory Siglec-5-like molecule while macrophages of wild-type homoygotes express only the activating Siglec-14. Macrophages of heterozygotes express both Siglec-5 and Siglec-14 [27,31].

Given the central role of macrophages in controlling Mtb infection and the impact of the SIGLEC14 genotype on myeloid cell function, we hypothesized that the SIGLEC14 null allele influences TB susceptibility in humans. We examined a South African pediatric cohort with TB disease and two Vietnamese adult cohorts, one with pulmonary TB (PTB) and the other with TB meningitis (TBM). We also evaluated SIGLEC14-dependent ex-vivo T-cell cytokine responses to Bacillus Calmette Guerin (BCG) vaccine to determine if the molecule plays a role in the development of the adaptive immune response. Lastly, we compared Mtb replication in a monocyte cell line overexpressing either the inhibitory Siglec-5 or the activating Siglec-14. We hypothesized that the SIGLEC14 null allele is associated with Mtb replication in monocytes, T-cell responses to BCG, and clinical susceptibility to TB in children and adults.

2. Materials and methods

2.1. Human subjects recruitment

2.1.1. Vietnamese adult cohort

HIV-uninfected adults (age > 15 years) with PTB were recruited in Ho Chi Minh City, Vietnam, from a network of district tuberculosis clinics or from the Pham Ngoc Thach Hospital for Tuberculosis and Lung Diseases. Subjects had positive sputum smears for acid-fast bacilli and also met the following criteria: no history of previous tuberculosis treatment, no evidence of extrapulmonary or miliary tuberculosis, and negative HIV testing.

HIV-negative adults with TB were recruited from 1997 through 2008 from two hospitals in Ho Chi Minh City: the Pham Ngoc Thach Hospital for Tuberculosis and the Hospital for Tropical Diseases. Subjects were diagnosed with TB using the following two sets of criteria: “Definite TB” was defined as clinical meningitis (nuchal rigidity, abnormal CSF parameters) and positive Ziehl-Neelsen stain or positive Mtb culture from the cerebrospinal fluid. “Probable TB” was defined as clinical meningitis plus one or more of the following: chest radiograph consistent with active tuberculosis, acid-fast bacilli found in any specimen other than CSF, or clinical evidence of extrapulmonary tuberculosis. Severity of TBM was assessed at presentation using the British Medical Research Council TBM grade, the Glasgow Coma Scale, and the presence of a focal neurologic deficit (defined as the presence of cranial nerve palsy, monoplegia, hemiplegia, paraplegia, or quadriplegia) [32]. A subset of patients with TB had cytokine and chemokine levels measured from the CSF as previously described [33]. All patients with TB were followed up until the end of anti-tuberculosis treatment.

Controls consisted of umbilical cord blood from newborns at Hung Vuong Hospital, Ho Chi Minh City. All subjects were unrelated and >99% were of the Vietnamese Kinh ethnicity. Written, informed consent was obtained from patients or their relatives for the cord blood samples and if the patient was unable to provide consent. All protocols were approved by human subject review committees at the Hospital for Tropical Diseases, Pham Ngoc Thach Hospital, Health Services of Ho Chi Minh City, Hung Vuong Hospital, Oxford Tropical Research Ethics Committee, and the University of Washington. Subjects from this cohort have been used in other candidate gene association studies as previously described [34–41].

2.1.2. South African pediatric cohort

Study participants were enrolled by the South African Tuberculosis Vaccine Initiative (SATVI) at field sites in Worcester, South Africa [42–45]. This region has one of the highest incidences of pediatric TB disease in the world [46]. The cohort used in the current study is part of a larger BCG vaccination correlates of risk project with 11,680 infants [43,46]. Enrolled infants were vaccinated with BCG at birth, as is standard practice in South Africa. A nested genetics case-control study was performed to identify cases and controls during a 2-year prospective observation period. The cases and controls included those from Cape Mixed Ancestry (CMA) and Black African descent. The CMA ethnicity represents the genetic admixture of Khoesan, Black African, European and both east and south Asian populations that has existed for over 350 years [47,48]. Several terms have been used to describe CMA including South African Mixed Ancestry and “Coloured”, a collective term for people of mixed ancestry in southern Africa, which is an officially recognized census term in South Africa and routinely used for self-classification.

The criteria for the case definition of TB disease has been described previously [46]. Community-wide passive surveillance
systems identified patients with TB disease and children with symptoms concerning for TB disease. Briefly, all children with symptoms consistent with TB disease or who had contact with an adult with TB disease were admitted to a dedicated research ward for examination, chest imaging, tuberculin skin testing, two early-morning gastric aspirates, and two induced sputa for Mtb smear and culture. Subjects were described as “definite TB” if they had a positive Mtb culture, smear, or PCR from one of their samples. Subjects were described as “probable TB” if they had a chest radiograph consistent with or suggestive of TB in addition to one or more laboratory or clinic features (smear negative, cough >2 weeks, PPD skin test ≥ 15 mm, failure to thrive, and recent weight loss). Subjects diagnosed with TB by the treating physician and with 2 or more clinical features suggestive of TB but without consistent chest radiography were described as “possible TB”. All others were described as “not TB”.

Two groups of controls were included in our analysis, household contact controls and community controls. Household contact controls (HHCs) were study participants who lived in the same household as an adult with active TB disease but who themselves did not develop TB disease over the study period. Community controls had no history of TB disease and were not enrolled until they were at least 2 years old. Both HHCs and community controls were unrelated to cases.

Exclusion criteria included HIV positive infant or mother, BCG vaccine not administered within 24 h of birth, significant perinatal complications in the infant, any pre-existing acute or chronic disease in the infant, or clinically apparent anemia. For community controls, household contact with any person with TB disease or person who was coughing was an additional exclusion criteria. Parents or legal guardians of study participants were informed of the risks and benefits of study participation and signed informed consent prior to enrollment. The protocol was approved by the University of Cape Town Research Ethics Committee and the University of Washington Institutional Review Board.

2.2. SIGLEC14 genotyping

Genomic DNA was prepared from peripheral blood or buccal cells using the QiAmp DNA Blood or Blood and Tissue Kit (Qiagen). Genotyping for the SIGLEC14 null allele was performed using the TaqMan Copy Number Assay (ThermoFisher Scientific, Assay HS03319513_cn) on an Applied Biosystems Step One Plus Real Time PCR machine. For quality control, we confirmed approximately 15% of TaqMan Assay results by PCR with primers designed to amplify the SIGLEC14 wild type or null allele as previously described [27]. We found >98% agreement between the TaqMan Copy Number Assay and standard PCR. Samples for which the assays gave discrepant genotype results were not included in the analysis.

2.3. M. tuberculosis genotyping

For a subset of Vietnamese patients with PTB or TBM, Mtb was isolated and genotyped as previously described [49]. Briefly, bacterial DNA was extracted from cultures on Lowenstein-Jensen media. Isolates were genotyped by four established methods: IS6110 restriction fragment length polymorphisms (RFLP), spacer oligonucleotide typing (spoligotyping), 12 allele mycobacterial interspersed repetitive unit (MIRU) typing, and large sequence polymorphisms (LSP) defined by deligotyping. Phylogenetic trees were created using Bionumerics software [49].

2.4. T-cell cytokine assays

As a part of the larger SATVI project described above, whole blood was collected from participants at 10 weeks of age. Samples were then analyzed ex-vivo for secreted cytokine responses to BCG stimulation [42,43]. We stimulated whole blood with BCG for 7 h and subsequently measured IL-2, IFN-γ, IL-13, and IL-17 production using ELISAs.

2.5. Statistical analysis

We used STATA 11.2 (StataCorp) to conduct Pearson’s χ² testing as well as logistic regression on the case-control cohorts and Cox linear regression to evaluate mortality. To control for potential genetic heterogeneity in the CMA ethnicity, we included a set of 96 Ancestry Informative Markers (AIMs) for the complex five-way admixed South African Coloured population as previously described [50]. In CMA individuals, there were no significant differences in genotype frequencies of the AIMs between cases and controls. The AIMs were used to calculate a coefficient incorporating the first five principal components of the AIMs data, which accounted for over 60% of the variation in the dataset. We then used this data to create a regression coefficient for adjusting the primary case-control data for ethnicity by converting it into an ethnicity principal component coefficient using the “pca” command in STATA 11. This provided an alternative means of regressing for ethnicity within the CMA population. Results of South African peripheral blood cytokine assays were analyzed for association with SIGLEC14 genotype using a general linearized model in STATA 11. Vietnamese cerebrospinal fluid cytokine levels were compared to SIGLEC14 genotype using the Mann-Whitney U test.

2.6. Mtb replication in Siglec-5/14 overexpressing THP-1 cells

THP-1 macrophage-like cells stably overexpressing Siglec-5, Siglec-14, or transfected with empty plasmid have been described previously [27,29,51]. Overexpression of Siglec-5 and Siglec-14 mRNA relative to empty vector controls was confirmed by RT-PCR (data not shown). Transfected THP-1 cell lines were seeded in 96-well plates at a density of 100,000 cells/well. PMA was added at a final concentration of 50 ng/ml and cells were incubated at 37 °C overnight. Media containing PMA was subsequently removed and replaced with fresh media. Cells were then infected with Mtb, strain Erdman, that was transfected with a plasmid containing the LUX operon under the control of a Mycobacterial optimized promoter (gift of Dr. Jeffrey Cox) at a multiplicity of infection (MOI) of 5 or 10. With this system, luciferase expression, as measured by relative light units, serves as a proxy for colony count with a linear relationship over the levels of expression observed (data not shown).

Infections were incubated at 37 °C for 2 h, after which cells were washed x 1 with media and then replaced with fresh media. For each cell line, infections were performed in sextuplicate. As a background control, luciferase-expressing Mtb was also added to wells containing media alone (no mammalian cells). The background control wells were otherwise treated in the same manner as those wells containing mammalian cells. Luminescence readings were taken at day 0 and then daily from day 3 until day 7. Means of daily luminescence values, standard deviations, and two-tailed Student’s t tests were performed and graphed using GraphPad Prism Version 6.0.

3. Results

3.1. Association of the SIGLEC14 null allele with protection from tuberculosis disease in Vietnam

Using a case-population study design in Vietnamese adults, we
The SIGLEC14 null allele in 378 cord blood controls and 773 cases of TB disease (Table 1). The TB disease cohort was comprised of 380 cases of PTB without extrapulmonary involvement and 393 cases of TBM (together, labeled ‘AllTB’). The allele was in Hardy-Weinberg equilibrium in the control population ($\chi^2_p = 0.60$). The SIGLEC14 null allele was more common than the wild-type allele in the Vietnamese population, with a null allele frequency of 61%. We found a trend towards association between SIGLEC14 genotype and AllTB ($p = 0.063$, Pearson’s $\chi^2$ test with genotypic model). The association best fit a dominant genetic model with a lower frequency of individuals with the SIGLEC14 null allele in cases compared to controls ($p = 0.028$, OR = 0.69 for un-adjusted dominant model; $p = 0.032$ with OR = 0.65 when adjusted for gender).

We next looked for an association between SIGLEC14 genotype and the subgroups of PTB and TBM. We found a trend towards a significant association with TB (Table 2) and an association with either Siglec-5 or Siglec-14. The wild-type THP-1 cell line is heterozygous for the null allele based on our genotyping (data not shown). We also compared SIGLEC14 genotype with cerebrospinal fluid findings in patients with TB but did not find an association between genotype and any CSF markers including WBC count, neutrophil and lymphocyte differential, or CSF concentrations of TNF, IFN-$\gamma$, IL-1b, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, or IL-13 (sample size of approximately 100 individuals, data not shown). Lastly, the null allele was not associated with 9-month mortality (data not shown).

To determine if the SIGLEC14 null allele influenced the infecting strain of Mtb, we compared the Mtb lineage of isolates both from patients with PTB and TBM and compared for association with SIGLEC14 genotype (Table 2). We did not find an association with genotype and Mtb strain with either non-Beijing or Beijing lineage.

### 3.3. SIGLEC14 null allele and BCG-induced cytokine responses in South Africa

To examine how SIGLEC14 modulates susceptibility to TB disease, we considered adaptive and innate immune mechanisms in a South African pediatric cohort. We first examined whether SIGLEC14 influences BCG-induced cytokine responses. Following vaccination with BCG at birth, infants had blood drawn at 10 weeks of age to examine BCG-specific cytokine response by re-stimulating whole blood ex-vivo with BCG and measuring secretion of IL-2, IL-13, IFN-$\gamma$, and IL-17 (Fig. 1). Both IL-2 and IL-17 secretion were significantly higher with the null allele under a general linearized model ($p = 0.035$ and 0.006, respectively), though the IL-2 association does not remain significant when corrected for multiple comparisons (4 cytokines evaluated). In contrast, the SIGLEC14 null allele frequency was not associated with IFN-$\gamma$ or IL-13 levels. These data suggest that the SIGLEC14 null allele is associated with higher BCG-specific IL-2 responses and support a possible mechanism of protection from TB disease.

#### Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>WT/WT N (%)</th>
<th>WT/Null N (%)</th>
<th>Null/Null N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>378</td>
<td>55 (15)</td>
<td>172 (46)</td>
</tr>
<tr>
<td>AIITB</td>
<td>773</td>
<td>153 (20)</td>
<td>311 (40)</td>
</tr>
<tr>
<td>PTB</td>
<td>380</td>
<td>72 (19)</td>
<td>153 (40)</td>
</tr>
<tr>
<td>TBM</td>
<td>393</td>
<td>81 (21)</td>
<td>158 (40)</td>
</tr>
</tbody>
</table>

The SIGLEC14 null allele in Vietnamese adults with pulmonary tuberculosis (PTB) or tuberculous meningitis (TBM) compared to cord-blood controls from the same population. ‘WT’ indicates the wild-type allele, which contains the SIGLEC14 gene. ‘Null’ indicates the null allele, in which SIGLEC14 is deleted. Results of Pearson’s $\chi^2$ test are shown (Geno) as well as results of logistic regression for the dominant (Dom) model. The PTB and TBM cohorts were combined (AllTB) for the same analysis. Bold indicates $p < 0.05$. 
unpaired Student’s t test). A multiplicity of infection of 5 versus 10 yielded similar results. We did not observe a consistent difference between Mtb replication in THP-1 cells overexpressing Siglec-5 relative to Siglec-14. Together, these data demonstrate that overexpression of either Siglec-5 or Siglec-14 yields greater Mtb replication in-vitro than background expression in a THP-1 monocyte-like cell line.

4. Discussion

In Vietnamese adults, we found that the SIGLEC14 null allele was associated with protection from disease in the combined PTB and
null allele. Since Siglec-5 and Siglec-14 are expressed primarily on granulocytes and monocyte/macrophage lineages and only minimally expressed on T-lymphocytes [27,31], we hypothesized that Siglecs could regulate antigen presentation in dendritic cells and modulate T-cell responses to infection. The increase in BCG-induced secretion of the pro-inflammatory IL-2 in the presence of the null allele is partially consistent with an expected model of higher TH1-type adaptive immune responses leading to protection from TB disease [56]. Since null-allele macrophages express less of the pro-inflammatory Siglec-14 relative to the anti-inflammatory Siglec-5, it may be counterintuitive that the null allele would be associated with higher levels of the TH1 and TH17-type T-cell cytokine since we would expect T-cells to be activated by pro-inflammatory macrophage and dendritic cell signals. However, we previously found that TLR1/6-deficient individuals (defined by single nucleotide polymorphisms that regulate signaling in monocytes) had increased BCG-specific TH1 T-cell polarization and IL-2 secretion in the same cohort [42]. The hypo-responsive TLR polymorphisms were associated with decreased IL-10 and a shift in the ratio of IL-10 to IL-2 which could lead to increased TH1 polarization. A similar mechanism might explain our findings with Siglec-5 and Siglec-14. Expression of these two Siglecs is less well characterized in dendritic than in macrophages. Siglec-5 expression has been observed in dendritic cells differentiated from circulating monocytes in-vitro and from plasmacytoid dendritic cells from peripheral blood [57], though this observation was made prior to the discovery of SIGLEC14 and the recognition that most antibodies against Siglec-5 are cross reactive with Siglec-14. An additional consideration is that DAP12, to which the Siglec-14 cytoplasmic domain is complexed, might have a suppressive function in DCs; prior evidence suggests the dual functionality of this adapter molecule in different cell types [38]. Whether Siglec-5/14 regulation of DC function modulates BCG-specific adaptive immune responses requires further investigation.

Regarding our in-vitro live Mtb replication assays, we initially hypothesized that overexpression of the inhibitory Siglec-5 would result in increased replication of Mtb relative to control lines and that overexpression of the activating Siglec-14 would reduce Mtb replication. This hypothesis would be consistent with recent data showing more Group-B Streptococcus (GBS) growth in the inhibitory relative to the activating Siglec cell line [29]. While overexpression of Siglec-5 allowed for greater Mtb replication, so did overexpression of Siglec-14. Our in-vitro results were not consistent with those seen for GBS, nor were they consistent with our finding of increased protection against TBM in Vietnam in the presence of the SIGLEC14 null allele. One explanation for the in-vitro finding is that Mtb may use the extracellular domains of either Siglec molecule to gain entry into the cell in a manner that does not induce Siglec signaling. Overall, the in-vitro data derived from a manipulated cell line likely does not sufficiently encompass the complexity of the in-vivo relationship between the SIGLEC14 null allele and TB disease.

A limitation to our genetic analyses is the potential for population admixture in either of our cohorts. The SIGLEC14 null allele genotype frequencies in the self-reported Cape Mixed Ancestry
control population displayed borderline Hardy-Weinberg equilibrium, which suggests either population admixture or genotyping error. The latter possibility is unlikely as we verified our genotyping data with two independent methods for a subset of samples. To control for population admixture in South Africa we used principal components of ancestry informative markers. Even with this adjustment, the data remained non-significant. The Vietnamese population is more homogeneous (>99% Khinh ancestry) with no evidence of significant population admixture [59]. A potential confounder with the Vietnamese cohort is the use of cord blood as a control, which could lead to misclassification of controls that eventually become cases. Although this is possible, the misclassification would be low and correction would likely strengthen our observed association. A further potential limitation in our analysis is the possibility of case-control misclassification, particularly in our pediatric TB cohort given the challenges associated with diagnosing pediatric TB. To avoid misclassification we performed a sensitivity analysis in which we excluded possible and probable TB cases and only compared definite TB cases to controls. This did not alter the outcome of the analysis (data not shown). Additionally, our South African cohort is to our knowledge the largest genetic cohort of pediatric TB worldwide and diagnoses are made by experienced clinicians in a region with one of the highest densities of TB in the world.

The presence of the SIGLEC14 null allele varies widely across different populations worldwide. Prior genotypic data suggests that the null allele frequency is greatest in Chinese and Southeast Asian populations, followed in order of decreasing frequency by Middle Eastern, Sub-Saharan African, and Northern European populations [27]. Our results provide SIGLEC14 genotyping data for the largest Vietnamese and South African populations published to date. Our allele frequencies were consistent with those seen in prior studies, with a null allele frequency of 30% in our South African control population and 63% in our Vietnamese control population. To date, the clinical significance of this genetic event with potentially dramatic phenotypic consequence has only been explored in a small number of studies. Recent work found that the SIGLEC14 null allele was associated with reduced risk of COPD exacerbation [31]. The authors showed that nontypeable *Haemophilus influenzae*, a common cause of COPD exacerbation, can bind Siglec-14 and induce an inflammatory cascade that may be responsible for the increase in exacerbations in wild-type patients. On the other hand, Siglec-5 and Siglec-14 are expressed in fetal amniotic tissue as well as on leukocytes and the SIGLEC14 null allele was associated with the increased incidence of premature delivery in GBS-positive mothers [29]. Our genotyping data from Vietnam suggests that the differential expression of Siglec-5 or Siglec-14 plays a role in susceptibility to TB. Further mechanistic evaluation will be required to characterize the interaction between Mtib and Siglec molecules.

Acknowledgements

We would like to thank the participants in the study. We would also like to thank the immunology and clinical teams at the SATVI research site in Worcester and the Hospital for Tropical Diseases and Pham Ngoc Thac Hospital in Vietnam for obtaining informed consent and collecting and processing blood from the study participants. This research was supported by NIH 5T32HL007287 (ADG), NIH K24 AI089794 (TRH), NIH NOI-Al-70022 (Tuberculosis Research Unit) (TRH, WAH), the Burroughs Wellcome Foundation 1008461 (TRH), and the Dana Foundation (TRH and WAH).

References

clinical benefit of adjunctive dexamethasone in tuberculous meningitis is not associated with measurable attenuation of peripheral or local immune responses. J Immunol 2005;175(1):579–90.


