Mediastinal Fibrosis Is Associated With Human Leukocyte Antigen-A2*

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Objective: To determine the association between mediastinal fibrosis and human leukocyte antigen (HLA) genes.

Design: Case-control study.

Setting: Vanderbilt University Medical Center.

Subjects: Nineteen consecutive patients with mediastinal fibrosis who presented to the pulmonary clinic at Vanderbilt University Medical Center from 1987 to 1996. The control subjects were 21,086 whites who were cadaveric kidney donors from October 1987 through December 1993.

Measurements: HLA testing was performed on blood samples from all 19 cases. Information on HLA typing for the control subjects was obtained from the United Network for Organ Sharing. Frequency of HLA class I and II antigens found in the cases was compared with the frequency in the control subjects.

Results: The relative risk of mediastinal fibrosis among subjects with the HLA-A2 antigen was 3.32 times that of those who lacked this antigen (95% confidence interval, 1.19 to 9.2).

Conclusion: HLA-A2 was strongly associated with mediastinal fibrosis, suggesting that an abnormal immune response is important in the pathogenesis of this disease.

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Key words: Histoplasma capsulatum; human leukocyte antigen-A2; mediastinal fibrosis

Abbreviations: HLA = human leukocyte antigen; MHC = major histocompatibility complex

Mediastinal fibrosis is characterized by excessive, perinodal fibrotic proliferation that invades and destroys normal mediastinal structures. Dense fibrotic proliferation arises from nodal locations that are believed to be residua of remote mediastinal adenitis, as evidenced by the caseous focus often found at its center. Remote infection with *Histoplasma capsulatum* accounts for the majority of cases of mediastinal fibrosis in the United States, with diagnosis usually based on special stains revealing the organism in tissue in up to half the patients, but rarely by complement fixation or culture. No other etiologic agent for mediastinal fibrosis has been reported, and there have been no distinguishing features that differentiate between the cases caused by histoplasmosis and those in which *H. capsulatum* was not found. Even in areas where the organism is endemic, mediastinal fibrosis remains an uncommon complication of histoplasmosis, occurring in perhaps 1 in 100,000 of the population. Most individuals who reside in endemic areas are infected with Histoplasma from time to time, and the reason a very small minority develop this serious process is unknown. The exuberant fibrosis in combination with the rarity of the disease raise the question of whether affected patients have an abnormal immunologic response to *H. capsulatum*.1,2

Several immunologically mediated diseases have been associated with specific human major histocompatibility complex (MHC) molecules. MHC class I molecules (human leukocyte antigen [HLA]-A, HLA-B, and HLA-C) are cell surface molecules located on almost all nucleated cells and are involved in presenting antigen peptide fragments to CD8+ T lymphocytes.3 The peptide fragments presented by MHC class I molecules are predominantly derived from the intracellular environment, in contrast to the extracellular antigens presented by MHC class II molecules (HLA-DP, HLA-DQ and HLA-DR) to CD4+ T cells. However, recent studies reveal that some MHC class I molecules may also present...
antigens derived from exogenous proteins. We sought to identify associations between HLA molecules and mediastinal fibrosis that might lead to understanding of the development of this disease.

**Materials and Methods**

**Participants**

Nineteen consecutive patients with mediastinal fibrosis who presented to the pulmonary clinic at Vanderbilt University Medical Center from from 1987 to 1996 were studied. Blood samples for HLA typing were collected during a phlebotomy that was indicated for clinical purposes. Typing for HLA class I (HLA-A and HLA-B) and II (HLA-DR) antigens were performed for 16 patients. For the remaining three patients, only HLA class I typing was done. The study was approved by the Institutional Review Board of Vanderbilt University Medical Center, and informed consent was obtained from each participant. The control subjects were 21,086 white, cadaveric kidney donors for whom HLA typing for HLA-A, HLA-B, and HLA-DR antigens was available through the United Network for Organ Sharing database for the time period October 1987 to December 1993.

**HLA Typing**

Typing in mediastinal fibrosis was performed using standard National Institutes of Health technique for cytotoxicity testing by Dialysis Clinics Inc. laboratories. First, 1 μL of leukocyte preparation (2 × 10^5 mL) was incubated with 1 μL of reagent typing sera and incubated at room temperature for 30 min. Next, 5 μL of rabbit complement was then added, and the typing tray was incubated for an additional 60 min at room temperature. An eosin-based stain fixative was then added, and the cells were examined microscopically using dye-exclusion to determine viability.

**Statistical Methods**

The frequencies of HLA class I antigens in the patients with mediastinal fibrosis were compared with those in the control group. The relative risks of mediastinal fibrosis in subjects with different HLA phenotypes were estimated by odds ratios, and 95% confidence intervals for these risks were estimated by Woolf’s method. The statistical significance of the relative risks was assessed using the χ² test with Yates’ correction.

**Results**

Of the 19 patients, 13 were female and 6 were male. Eighteen were white, and 1 was African American. The mean age (± SEM) at the time of diagnosis of mediastinal fibrosis was 29.4 ± 2.7 years. Table 1 shows the anatomic structures compromised by the fibrotic process in these patients.

Among the 19 patients with mediastinal fibrosis, 32 MHC class I haplotypes were found. A positive association was found between mediastinal fibrosis and HLA-A2. Fourteen of the 19 patients (73.7%) had the HLA-A2 antigen vs 9,642 of the 21,086 control subjects (45.7%). Thus, the relative risk of mediastinal fibrosis was 3.32 times greater (95% confidence interval, 1.19 to 9.23; p = 0.027) in subjects with the HLA-A2 antigen than in those who lacked this antigen. No other statistically significant associations were found between mediastinal fibrosis and HLA class I antigens.

The study of MHC class II haplotypes was restricted to HLA-DR antigens. Ten HLA-DR haplotypes were found among the 16 patients with mediastinal fibrosis for whom MHC class II typing was performed. A significant association was found between mediastinal fibrosis and HLA-DR53. However, we feel that this association is unlikely to be clinically important, as the statistical significance was based on the presence of the antigen in only one of the patients with mediastinal fibrosis and none of the 21,086 control subjects.

**Discussion**

Mediastinal fibrosis is rare, but it is the most life-threatening sequela of infection with *H capsulatum*. Mediastinal fibrosis as a late complication of...
Histoplasmosis is a focal, invasive, calcified process that has not been shown to respond to any therapy. In contrast, many individuals who reside in endemic areas have fibrotic calcified nodes that are of no clinical importance.\(^1\) *H capsulatum* derived its name from Darling’s description of the organism as a plasmodium-like structure within histiocytes.\(^7\) The organism is found as a mold in soil, but at body temperatures, it grows as a yeast. With low level exposure to *H capsulatum*, only about 1% of persons will develop self-limited disease with flu-like symptoms, while the remaining 99% will be asymptomatic. Rarely, patients with exposure to the organism will have a chronic inflammatory disease resulting in mediastinal fibrosis, a condition that frequently presents with shortness of breath, chest pain, and hemoptysis.\(^8\) Our definition of mediastinal fibrosis was partial or complete occlusion of a major airway or a great vessel (pulmonary artery or vein or superior vena cava) by the fibrotic process.

The causes and mechanisms of mediastinal fibrosis, of which there may be several, are poorly understood. A distinctly different form of mediastinal fibrosis not included in this study is characterized by extensive, diffuse, noncalcified encasement of the trachea.\(^9\) We diagnosed only one case of this very rare form of mediastinal fibrosis during the course of the study. This form of the disease may be steroid-responsive and is probably best classified with diseases like retroperitoneal fibrosis.

The rarity of mediastinal fibrosis in areas where histoplasmosis is endemic suggests that an abnormal host response to infection with this organism may lead to excessive fibrosis. The initiation of the immune response begins with antigen presentation. We sought to determine whether specific MHC molecules involved in antigen presentation might play a role in the pathogenesis of mediastinal fibrosis.

MHC class I molecules are assembled in the endoplasmic reticulum. In the endoplasmic reticulum, the MHC class I molecules associate with the transporter associated with antigen processing, a protein that processes and transfers protein fragments from the cytosol into the endoplasmic reticulum. These protein fragments, or peptides, can then be captured into the MHC class I binding groove and then transported to the cell surface. Once at the cell surface, the MHC class I–peptide fragment complex can interact with CD8+ T cell receptors, initiating an immune reaction. The antigen receptor of a given CD8+ T lymphocyte recognizes antigen peptide fragment only within the context of the specific class I molecule, a phenomenon known as MHC restriction. Once the MHC class I–peptide fragment complex initiates an immune response via the CD8+ T cell receptor, cells expressing the same MHC class I–peptide fragment complex become cytotoxic targets for clones of these CD8+ T lymphocytes. This limitation in the generation of cytotoxicity based on restriction of antigen presentation to a specific class I molecule provides an explanation for the linkage of MHC class I molecules to various disease states.\(^3\) In addition, CD8+ cells, when stimulated by antigen presentation, can produce a variety of cytokines that can further propagate an immune response.\(^10\)

Several diseases have been associated with the MHC class I molecule HLA-A2. These include rheumatoid arthritis, juvenile rheumatoid arthritis, psoriatic arthritis, Alzheimer’s disease, hepatitis C, nasopharyngeal carcinoma, and Epstein-Barr virus–associated posttransplantation lymphoproliferative disorder after lung transplantation.\(^11–16\) Four hypotheses have been proposed to explain the link between specific MHC class I molecules and certain disease states.\(^17\) One is that specific class I molecules may act as receptors for disease agents such as viruses, toxins, or other foreign substances. Another is that the peptide fragment binding groove of only a particular MHC class I molecule can bind the processed antigenic peptide fragment that is responsible for causing disease. A third hypothesis is that the T cell receptor is responsible for causing disease; however, the ability of a T lymphocyte to “see” a specific antigenic peptide complex is related to the specific MHC class I molecule in which the peptide fragment is bound. A fourth possibility is that the MHC class I molecule may be immunologically similar to a disease-causing agent and that the immune response generated against the disease is also mounted against the similar MHC class I molecule.

In addition to these four possible explanations for an association between immunologic disease and specific MHC class I haplotypes, new evidence suggests a fifth mechanism by which there is a link between extracellular pathogens and MHC class I antigens. Until recently, the general consensus among immunologists was that MHC class I molecules solely presented endogenous antigens to CD8+ cytotoxic T lymphocytes.\(^3\) However, cytotoxic T lymphocyte responses have now been reported to bacteria and parasites that never enter the cytosol.\(^18\) The mechanism by which these extracellular organisms enter the MHC class I binding groove is unknown. One hypothesis is that antigens from these organisms escape from phagocytic lysosomes and can then be processed through the transporter associated with antigen processing to be bound in the MHC class I binding groove, thus stimulating a CD8+ T lymphocyte response.

The mechanism by which exposure to an organism that causes asymptomatic disease in a large propor-
tion of the population leads to severe disease in such a small minority is unknown. The association we found between an MHC class I molecule, HLA-A2, and mediastinal fibrosis provides insight into possible disease pathogenesis. Future technical developments may allow for analysis of peptides within the HLA-A2 binding groove that may be disease-causing, or identification of CD8+ T cell clones that could be eliminated to cease disease progression.

Clinicians have wondered whether a new method might be found to identify individuals at risk of developing mediastinal fibrosis so that strategies to prevent the disease might be employed. Although the predictive value of the HLA-A2 association with mediastinal fibrosis is poor because of the high prevalence of HLA-A2 in the general population, this finding surely supports the concept that underlying individual susceptibilities may be operative, and that further studies might provide a test of greater clinical predictive value.

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