Asthma remains one of the major respiratory diseases in the world, affecting millions of humans each year. With its prevalence in Westernized, developed countries steadily increasing over the last 25 years, asthma will continue to be a major focus of respiratory research in the future. The causes of this increase remain unknown, but both genetic and environmental sources have been blamed. It has also been noted that children who contract respiratory infections at a young age have a much lower prevalence of asthma later in life. But oddly, similar children who are vaccinated against influenza do not retain this same immunity. Early infection, and the consequent altering of the immune system, could be the key to asthmatic immunity. This alteration occurs at the cellular level, meaning that the molecules that signal and bind immune system cells to the lung during asthma are the crucial determining factors in the mounting of an immune response. Other studies have shown that children in daycare and children in large families have a lower incidence of asthma, possibility due to greater exposure to respiratory viruses that would modify their immune systems. A vaccine to help prevent asthma attacks would be very useful and may now be possible, as studies of these fundamental adhesion molecules, which change the immune system’s response to the disease, are highly promising.

Asthmatic symptoms, such as restricted breathing and airway inflammation, can be linked to two specific cytokines (chemical signals), Interleukin-4 (IL-4) and IL-5. IL-4 induces Immunoglobulin E (IgE) synthesis, mast cell activation, and eosinophil recruitment. Immunoglobulins, commonly called antibodies, are the proteins involved with antigen recognition and cell-cell interaction. IgE levels correlate directly to asthma, and it has been shown that IgE induces the acute phase of asthma. The acute phase occurs 2-5 minutes after exposure to an allergen and involves a non-specific process, such as histamine, to induce a very broad, general response. The chronic phase occurs later and involves more specific cells, such as CD4+ T cells and B cells, which are sometimes recruited as memory cells if the allergen has previously been detected in the body. The findings described in this article pertain to the chronic phase for asthma.

Mast cells activated by IL-4 produce histamine (an inflammatory molecule) and IL-5. Eosinophils respond to an allergic asthma reaction, and are thought to be important chiefly in the defense against parasitic infections. The airway inflammation caused by IL-5 and histamine molecules leads to hyperreactive Airways and bronchoconstriction. This reduction in airway diameter causes the common symptoms of coughing and wheezing, and also means the airway is much more sensitive to small amounts of the specific allergen. An increase in the secretion of mucus makes breathing difficult by trapping inhaled air in the lung. Mice that lack the gene for IL-4 production (called IL-4 knockout mice) have no inflammation in the lungs and little airway hyperreactivity, proving the cytokine’s important role in the asthmatic response. IL-5 has a different, but nonetheless important, role in producing the symptoms of asthma. IL-5 induces eosinophil differentiation and activation. Studies done with IL-5 knockout mice, lacking IL-5, had similar results to the IL-4 knockout experiments. The mice were shown to have reduced airway hyperreactivity, lung damage, and eosinophil levels. However, there was no decrease in IgE production, which suggests that IL-5 has no role in the activation of IgE.

Allergic asthma is caused by the inhalation of a simple allergen, usually a common, non-toxic protein found in nature. In mice, chicken Ovalbumin protein (OVA) has been used successfully as an allergen to model allergic asthma. Mice were first immunized with two very dilute OVA injections in the abdominal cavity 7 days apart. After 7 more days, they were challenged with a much stronger dose of OVA (40mg/ml of saline) inhaled through the nose. The immune response was then measured on day 5 after the challenge, which has been proven to be the peak time point of the CD4+ T cell allergic response.

CD4+ T helper cells are the major lymphocyte responders in an allergic response and are the main cells responsible for the memory of an allergen. CD4+ T cells are activated by short segments of the Ovalbumin protein, called antigens, and migrate to the lung in an attempt to destroy the allergen by activating macrophages and B cells. These allergen-specific CD4+ T helper cells produce IL-4 and IL-5, and are thus very important in the study of allergic asthma.

The adhesion molecules responsible for CD4+ T cells’ attachment to the extracellular matrix of the lung during allergic...
asthma are still relatively unknown. However, recent studies point to Very Late Antigen 1 (VLA-1) and VLA-2 as likely candidates. These two heterodimeric integrin adhesion proteins both appear very late after T cell activation, with VLA-1 specifically binding to Type IV collagen on the extracellular matrix and VLA-2 binding to Type I collagen. Both of these types of collagen are found in the lung. Recent studies show that VLA-1 is the pivotal adhesion molecule in the retention and survival of CD8+ T cells recruited to flu infections in non-lymphoid organs, such as the lung. But VLA-1 and VLA-2’s role in the retention of CD4+ T cells during allergic asthma has yet to be established.

The expression of these adhesion molecules could be the vital factor in the ability of the immune system to mount a successful memory response against asthma-inducing allergens. With this knowledge, these T cells’ adhesion molecules could be altered, leaving them unable to bind to the lung, thus causing little damage during an allergic response. This development would significantly reduce the effects of asthma, and in some cases even eliminate the disease.

In a typical immune response, lymphocytes are initially present in the lymph nodes and spleen and not the periphery tissues, such as the lung. Within the first 2 days after the challenge, however, the number of cells in the lymph nodes peak, as Antigen Presenting Cells present the OVA antigens to naïve T cells. These T cells (mostly CD4+) then travel to the site of infiltration, in this case the lung, peaking at day 5 of the response. Presumably, some cells begin to slowly die, apoptosing (actively committing suicide) as the OVA proteins become scarce. Some cells will also leave the site of inflammation and travel to other organs of the body, although the destinations, such as the liver and other periphery organs, and the recruitment of these cells are still being researched.

T cell populations in the lung and the BAL (a wash of the lungs) were predominantly CD4+ on day 5 after the initial OVA challenge (Figure 1). T cells in the lymph nodes (MLN) and spleen were unaffected by the influx in CD4+ cells in the lung (Figure 1). The higher than normal percentages of CD8+ cells observed outside of the lung (specifically in the MLN) can be attributed to the increase in growth factors such as IL-2 and IL-4, which stimulate both CD8+ cells and non-specific CD4+ cells. Thus, all of the lymph nodes will not have elevated CD4+ populations, while the CD8+ cells will be slightly increased. Due to its large size, the spleen sees little change in either cells’ population.

EliSpot assays were performed in order to check the specificity of these cells, and to ensure that they were truly specific for and induced by the OVA allergen. This assay attaches antibodies specific for the cytokine IL-5 to a filter. Live cells from the animal stimulated with OVA produced much more IL-5 to a filter. Live cells from the animal induced by the OVA allergen. Therefore these cells recognize OVA and are specific for it. Although the presence of OVA specific cells in the lung appears small (Table 1), a fraction of one percent of T cells in an organ is, in fact, a significant population. If we consider that there are approximately 10^9 different T cell receptors present in the human body, populations 0.11% and 0.23% of all CD4+ cells are not insignificant. On average a given specificity of T cells in a mouse only constitutes 0.001% of the total population.

Having answered the question of specificity, we now move ahead to the expression of the adhesion molecules VLA-1 and VLA-2 on these OVA specific CD4+ T cells. BAL and lung CD4+ T cells from day 5 mice were stained to show the expression of VLA-1 and VLA-2 adhesion molecules. The CD4+ cells in the BAL expressed more surface VLA-2 (62.89%) than VLA-1 (19.26%) (Table 1). A significant population (15.70%) of these CD4+ cells also expressed both integrins. CD4+ T cells in the lung showed a similar pattern of expression, although the CD4+ cells were present at a lower percentage (Figure 2). However, this does not completely rule out VLA-1’s importance in the retention of CD4+ cells; although, due to its low

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**Figure 1.** Populations of CD4+ and CD8+ T cells in four organs of day 5 OVA induced asthmatic mice. CD4+ cells can be located in the upper left quadrant, while CD8+ cells can be found in the lower left. Numbers indicate percentages of the cells. The larger difference between CD8+ and CD4+ T cell populations in the BAL was expected as CD4+ T cells are heavily recruited to the site of inflammation, and are the main responders in an asthmatic attack. CD8+ T cells remain inactivated in the lymph nodes, but are increased due to the presence of the cytokine IL-2, which is produced by the proliferating CD4+ cells. Interestingly in the lung, the CD4+ population is not as great, but is still much greater than the CD8+ population.
expression, it is doubtful that VLA-1 plays a major role in CD4+ retention. Retention of the T cells in the lung cannot be made by simply comparing the percentages of the loose cells of the BAL to those "stuck" in the lung, as one might guess. Presently we do not know the exact strength with which the BAL tears adhered cells away from the extracellular matrix, so we assume that the BAL cells were previously attached in some way to the lung.

Initial experiments, where influenza (strain A/HK/x31) was used to induce a CD4+ T cell response concur with these findings on VLA-1 and VLA-2 expression. At the peak of an influenza response (day 8), the BAL CD4+ T cells showed significant expression of VLA-2 (62%), while a less substantial population expressed VLA-1 (41%), and 30% of the population expressed both integrins.9 This suggests VLA-1 may play a more important role in activating CD4+ T cells during viral infections than it does in allergic asthma (due to the lower expression [41% compared to 19%] in the asthma model). In contrast, VLA-1 is the major integrin (66%) on CD8+ T cells in the influenza model.10 The differences between a viral infection (T helper type 1 reaction) and an allergic reaction (T helper type 2 reaction) likely explain the differences in the expression of what can be assumed to be the "minor" integrin, VLA-1. Alternatively, this may also reflect the difference between CD4+ and CD8+ T cells.

Thus, we have demonstrated the OVA specificity of significant populations of CD4+ T cells. We have also shown that CD4+ T cells express predominantly VLA-2 and, less significantly, VLA-1. However, the link between the two findings, that the OVA specific CD4+ T cells are the same cells that are expressing mainly VLA-2, still needs to be proven. One way to test this hypothesis is to transfer cells that are transgenic for the OVA antigen receptor into naïve mice where their expression of VLA-1 and VLA-2 can be tracked. This process can be used to determine if the OVA specific cells are in fact the same cells expressing these adhesion molecules. Additional experiments with VLA-1 and VLA-2 blocking antibodies will be performed to see whether airway hyperresponsiveness or allergic asthma is reduced with hindered expression of VLA-1, VLA-2, or both integrins.

Our research suggests that CD4+ T cells express predominantly VLA-2 during allergic asthma adhesion. We have also shown that very significant populations of OVA specific CD4+ T cells are found in the lung after an asthma response to OVA. CD4+ T cells are the most important and prominent cells found during and after an asthmatic attack. By understanding what holds a T cell in the lung (VLA-1 and VLA-2), scientists can find a way to alter this adhesion and to prevent T cells from causing damage due to inhalation of a harmless allergen. Certainly this adhesion pattern holds true for other types of infections whose main responders are CD4+ T cells, as suggested by similar data derived from a similar influenza model. Thus, increasing adhesion of these same cells could lead to stronger and quicker immune responses to more dangerous infections. Regardless of whether cell adhesion should be increased or decreased, this further understanding of cell surface molecules may prove invaluable in the reduction of the prevalence of respiratory illnesses worldwide.

David Meisenheimer, originally from Avon Lake, Ohio, graduated from the University of Rochester in 2003 with a B.S. in Microbiology and Immunology. He worked in Dave Topham’s (Ph.D.) lab for a year and a half where he performed research on asthma. He is a varsity cross-country runner and will attempt to make the Olympic Trials in 2004 as a triathlete. Afterward, he intends to pursue a Ph.D. in immunology or epidemiology.