Chapter

The Effect of Atmospheric Pollution on the Thymus

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Abstract

Air pollution is a high-risk factor in megacities' dwellers because of its effects on health. One of the most important components of the pollution is particulate matter (PM) on which metals are adhered. One element adhered to its surfaces is vanadium (V), and through this route, PM reaches the respiratory system, then the systemic circulation and the rest of the organs. Vanadium is released in the atmosphere as a consequence of the combustion of fossil fuels. Vanadium pentoxide is the compound liberated after the combustion and adhered into PM. Previous studies from our group have reported effects on diverse systems in a mouse model. Besides the morphological changes in the spleen and the decreased function of the immune humoral response, the thymus was also affected. Vanadium inhalation diminished thymic dendritic cells (DCs) and the biomarkers: CD11c and MHCII; in addition, thymic cytoarchitecture changed, demonstrated by cytokeratin-5, and also, modification in the expression of 3-nitrotyrosine was observed. Our findings suggest that autoreactive T cells could be released into the systemic circulation and favor the increase in autoimmune diseases in cities with high concentrations of PM.

Keywords: thymus, vanadium inhalation, dendritic cells, oxidative stress, nitrosative stress, autoreactive T cells

1. Introduction

The air is the source of a variety of pollutants such as gases and particulate matter (PM). Particulate matter sources are construction sites, unpaved roads, forest fires, volcanic eruptions, power plants and a variety of combustion processes. Internal combustion motors are an important source of PM, especially those with old technology and without the proper maintenance [1]. The PM size is linked to their capacity to produce health problems since the smallest can reach the deepest lung spaces, the alveoli, and translocate into the blood stream. Doing so, PM reaches diverse systems and organs producing physiological modifications. One of the systems affected by PM is the lymphoid. Few papers report the direct effect of the PM in the Thymus, which is a central actor in the future definition of the immune response and self-recognition and self-tolerance, increasing the risk for developing allergic or autoimmune diseases [2–6].

Dendritic cells play an important function as mediators between innate and adaptive responses and they are susceptible to the effect of some of the components of the PM, such as transition metals, especially vanadium (V) which is liberated

into the atmosphere by the combustion of fossil fuels [7]. One of the mechanisms by which V produces its effects is by oxidative or nitrosative stress and this mechanism is also proposed as the way by which the immune system is affected. In this report we describe the effect of vanadium, as a component of PM, and its oxidative and nitrosative effect on the structure and cells of the thymus.

2. Oxidative and nitrosative stress

Reactive oxygen and nitrogen species (RONS) are produced by cells normally as a result of their metabolism, and they function as key molecules in the maintenance of homeostasis by participating in various signaling pathways [8].

ROS include non-radical molecules derived from the molecular reduction of oxygen such as hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl) and oxygen-derived free radicals, such as: superoxide anion ($^{\cdot}O_2$) and hydroxyl radical ($^{\cdot}OH$) among others. The RNS include non-radical molecules such as nitrous acid (HNO_2), peroxynitrite (ONOO-) and free radicals derived from nitrogen such as nitric oxide ($^{\cdot}NO$) and nitrogen dioxide ($^{\cdot}NO_2$) among others [9, 10].

It is important to note that free radicals can be derived from many elements and molecules in addition to oxygen and nitrogen, such as hydrogen, carbon and transition metals such as iron and copper. RONS are important from the biological point of view due to their reactivity, which allows them to interact with different biomolecules [11, 12].

In the cells, reactive oxygen species are produced mainly through mitochondrial respiration, although there are other sources such as NADPH oxidases, microsomes, peroxisomes and other enzymes of metabolism such as CYP450 [11–13]. Reactive nitrogen species, such as $^{\cdot}NO$, are produced from the metabolism of L-arginine in a process catalyzed by nitric acid synthases (NOS). By combining the radical $^{\cdot}NO$ with $^{\cdot}O_2$, the anion peroxynitrite, which is reactive nitrogen species, can be formed [11]. The latter reveals the close relationship between the production of ROS and RNS.

Cells have physiological mechanisms that usually counteract the presence of reactive species by keeping them at low levels: antioxidants. These maintain the RONS levels below the toxic threshold. Under conditions in which the antioxidants are in imbalance with RONS, and the balance is tilted in favor of the latter, oxidative/nitrosative stress occurs [12, 14, 15].

As mentioned above, all species are able to interact with biomolecules (nucleic acids, proteins, lipids and carbohydrates), and under conditions of oxidative or nitrosative stress, RONS produce negative effects on them, altering their biological functions. Lipid peroxidation, protein modification and DNA oxidation are clear examples of the damage produced by the interaction with RONS [16].

External factors have been identified, such as the exposure to pollution, which may induce an increase in the production of RONS, causing oxidative and nitrosative stress [17]. Within the multiple components of the pollution, suspended particles and the metals, attached to them, increase the production of both types of species in the cells inducing oxidative and/or nitrosative stress. This stress has been associated with adverse effects such as inflammation, cytotoxicity and cellular damage in general [18]. It has been identified that soluble metals that are part of the particles such as iron (Fe), nickel (Ni), cobalt (Co), copper (Cu), chromium (Cr) and vanadium (V) generate these effects [19, 20].

What are the mechanisms involved in the formation of RONS by the participation of metals? The metals can induce the formation of free radicals through the Fenton reaction, in which the metal reacts with hydrogen peroxide (H_2O_2)

producing the hydroxyl radical ($^{\cdot}OH$) and an ion of the oxidized metal. It has been identified that metals such as cobalt (Co), chromium (Cr), nickel (Ni), iron (Fe) and vanadium (V) can be part of this reaction. Another mechanism by which metals produce free radicals is the Haber-Weiss reaction; in it an oxidized metal ion is reduced by superoxide ($^{\cdot}O_2$) and subsequently reacts with H_2O_2 producing hydroxyl radical. Metals such as cobalt, chromium and vanadium can participate in this reaction [21, 22].

The study of oxidizing and nitrosative stress is relevant due to the consequences that may have on biomolecules, and at a higher level, on the functions of organisms; this study can be approached with different methods.

One of the most used tools is the detection of products modified by reactive species, since they are more stable than the species themselves. Among the products that can be detected are those of oxidized lipids (such as aldehydes and ketones), proteins (as carbonylated and nitrosylated amino acid residues) and nucleic acids (such as 8-oxo-2-deoxyguanosine). An important utility of the ROS and RNS markers is their potential to identify the nature of the oxidant itself [9].

3-Nitrotyrosine (3-NT) has been identified as one of the most relevant markers, which shows the modification in proteins as a consequence of nitrosative stress. This marker is formed as a product of the nitration of tyrosine residues in proteins and occurs through the action of a nitrosative agent (ONOO-, $^{\cdot}NO$, HNO_2, etc.) that is added to the amino group (NO_2) of the polypeptide chain, leading to the nitration of tyrosine residues. This marker can be identified by immunoassays such as immunohistochemistry, immunofluorescence, ELISA [23].

It has been reported that 3-nitrotyrosine is involved in different pathological conditions such as inflammation, endothelial dysfunction, cardiovascular, liver, neurodegenerative, immunological diseases, aging, among others [23, 24].

3. Thymus and vanadium

The thymus is a capsulated primary lymphoid organ to which immature peripheral T-lymphocytes, from the bone marrow, arrives to complete its maturation and immune capacitation. It is located in the mediastinum; it has two lobules that originate from the third and fourth branchial poaches. In humans the thymus is fully formed and functional at birth and it reduces its size after puberty; however, it remains functional.

Histologically, it has a connective tissue capsule that extends into the parenchyma dividing it in incomplete lobules. In each lobule medulla and cortex are well delimited. The cortex is highly basophilic when the thymus is stained with hematoxylin and eosin as a consequence of the numerous and rapidly dividing immature T-lymphocytes called thymocytes, while the medulla is less basophilic because the thymocytes density decreases. Other cells in the thymus structure are the epithelial cells, located in the cortex-cortical epithelial thymic cells (cTEC)-, the medullary epithelial thymic cells (mTEC)-in the medulla-, also dendritic cells (DCs) located in the corticomedullary zone and in the medulla in addition of widely distributed macrophages. Small spherical-shaped structures, formed by mTEC, identified as Hassall's corpuscles -are thymic unique structures; its function is to regulate the production and maturation of the regulatory-T cells (Tregs). The thymus has a hematothymic barrier constituted by the vascular face of the endothelial cells from the cortex continuous capillaries, the basal lamina from the cortex continuous capillaries and the cTEC. Its function is to prevent the contact of the circulating antigens with the cortical thymocytes [25].

3.1 Positive and negative thymus selection

The maturation process from thymocytes to immunocompetent T cells depends on the cortical and medullar thymic microenvironments that is established by the cTEC, mTEC, and DC. The cTEC positively select the T-lymphocytes that recognize the major histocompatibility complex (MHC), so the selected T-lymphocytes are CD4+ or CD8+. The mTEC and the DCs are involved in negative T-cell selection and in establishing self-tolerance by eliminating through apoptosis, the lymphocytes which strongly recognize self-antigens, preventing autoreactive clones. Both processes area highly ordinated and rely on the sequential and quantitative expression of markers such as CD4, CD8, T-cell receptors (TCR1, TCR2) as well as of changes in the glycosylation of the membrane proteins on the lymphocytes in the process of maturation [26, 27].

3.2 Dendritic cells

Dendritic cells (DCs) are antigen -presenting cells (APCs) from the bone marrow. They are a heterogeneous population of cells which are identify because their surfaces biomarkers, cytokines production and location. Their progenitors could be myeloid or lymphoid. DCs are recognized by its typical dendritic morphology and by the expression of CD11c. As all of the other APCs, MHCII is expressed on its surface [28]. The differentiation and function of the DCs could be modulated by different growth factors and cytokines. There are three types: cDCs, the conventional type; pDCs, the plasmacytoid type; and mDCs, the monocyte-derived cells [29].

DCs are a bridge between innate and adaptive immune response. When they are immature its location is the skin and peripheral tissues and when an antigen is captured the cells migrate to present the antigen to the T cells. The thymus DCs and the mTEC are responsible of the thymocytes negative selection; in the medullae the single T cells (SP) interact with the DCs and with the mTEC that present them self-antigens with and immunogenic potential. The T-cell receptor (TCR) from the SP thymocytes recognizes the cells with MHC restriction and high affinity to self-antigens to eliminate them by apoptosis. This is the main mechanism to eliminate autoreactive clones and to establish central tolerance [29].

As it was previous mentioned V could damage health. Previous reports from our group demonstrated that, -in experimental model- mice that inhale V have damages in different systems including the immune system which induces changes in the cortex-medullae ratio [30, 31]. Other observed effect was in the spleen and in the humoral response [32].

Because of the morphological changes in the thymus as a consequence of the V-exposure, we decided to explore the changes in the expression of CD11c and MHCII on the DCs distribution.

We have developed a CD-1 mouse inhalation experimental model to assess systemic effects of PM. We used CD-1 mice (8-weeks-old, 33 + 2 g) from the vivarium of the School of medicine. The animals were randomly placed in acrylic chambers connected to an ultra-nebulizer (UltraNeb99 Devilbiss, Somerset, Philadelphia, USA), the exposure schedule consisted of V_2O_5 0.02 M (Sigma, St. Louis, Missouri, USA) in saline, 1 hour twice a week for 4 weeks. The ultra-nebulizer with the size of the particle emitted being less than 5 μm (range 0.5–5 μm) at a flow rate of 10 L/min. The concentration in the chamber was 1436 $\mu m/m^3$ [30, 31]. Mice were sacrificed at the end of each exposure week, the thymus was extracted and CD11c was detected by conventional immunohistochemistry. Other thymuses were processed for cytometry for CD11c+ and MHCII+. To obtain the enough cells the Baba method

was modified [33]. Briefly, mice were sacrificed, thymus were extracted, macerated and submitted to enzymic digestion to obtain a homogenate, by magnetic cell sorting (MACS) the CD11c+ and MHCII+, cells were separated. The cells from the 3rd week of exposure were selected for cytometry because previous result indicated that in this period of time thymus changes were more evident.

The observed changes in CD11c are shown in **Figure 1**. In controls more CD11c+ cells were noticed compared with the exposed mice. The findings were supported with the densitometric results (GraphPad Prism Software, V5, 2007, La Jolla, CA) that showed a diminished presence of the CD11c+ in the medullae as the time of the exposure progress.

Flow cytometry showed that levels of CD11c+ and MHCII+ were significantly decreased in the V-exposed mice as compared with controls. The loss was both in terms of number and in mean fluorescence intensity (MIF) values. In brief, V-inhalation decreased the thymic CD11C+ and MHCII+ cells as the exposure time increased [4]. An explanation for our findings could be the V-oxidative capacity which has been previously reported by our group and by other authors [34, 35]. Oxidative stress could affect the liberation of pro-inflammatory transcription factors by the CD11c cells. The thymus dysfunction could by in the negative selection of the T cells that, in turn, could alter the interaction of CD4 lymphocytes with the MHCII protein on the DCs. Such changes could then promote the development of autoimmune responses as suggested by Chang and indirectly by Zouali [36, 37], who hypothesized that an epigenetic effect from urban air pollutants led to altered immune cell phenotypes that, in turn, favors the development of autoimmunity. Further studies are planned to investigate this topic.

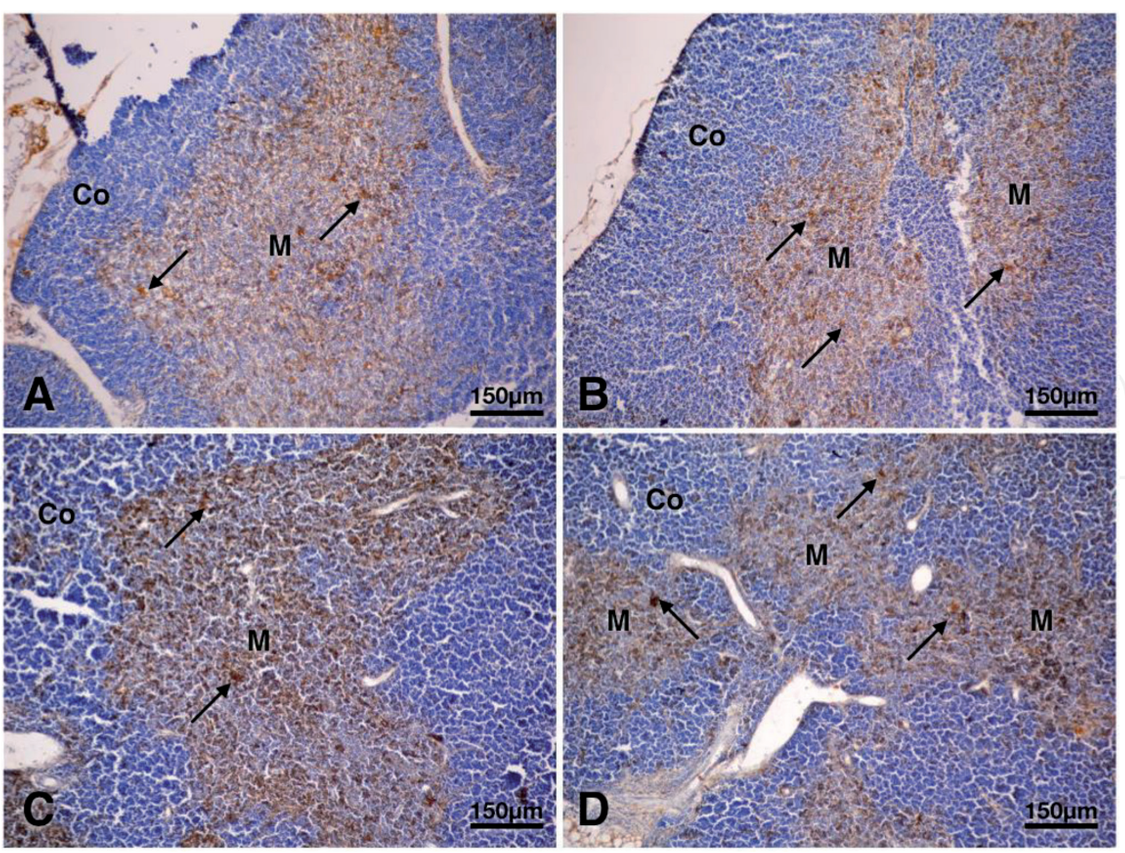

Figure 1.
CD11c expression in thymus. In control and V-exposed mice, ocher color stained (arrows) cells CD11c+ were distributed mainly in the medulla (M); scanty positive cells were present in the cortex (Co). Representative photomicrographs from (A, C) control (B, D) 4-week-V-exposed. (A, C) Reveal a higher presence of CD11c+ cells compared to the shown in (B, D).

3.3 Thymic epithelial cells

Previous studies have demonstrated the interdependence of TEC and the thymocytes to maintain the thymic microenvironment integrity. TEC are APCs distributed in the cortex (cTEC) and in the medullae (mTEC). cTEC select the thymocytes that express TCR and recognize self-MHC. The remaining cells are eliminated by apoptosis; this positive selection takes place in the cortex. mTEC with DCs are in charge of the negative selection of T cells and to establish central tolerance, as well as helping to regulate the production of regulatory T cells (Treg) [26, 27]. Previous studies from our group have demonstrated that V exposure change the thymic

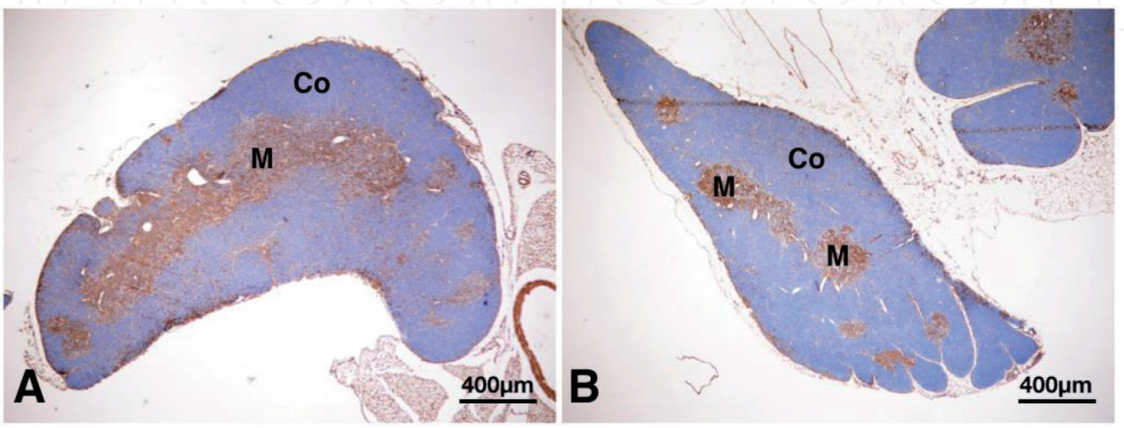

Figure 2.
K5 expression in the thymus. Representative photomicrographs from: (A) control and (B) 2-week-V-exposed. Positive K5 cells are located in the medullae (M) and some in the cortex (Co), B. K5+ cells located in the medulla (M) but also in the cortex (Co).

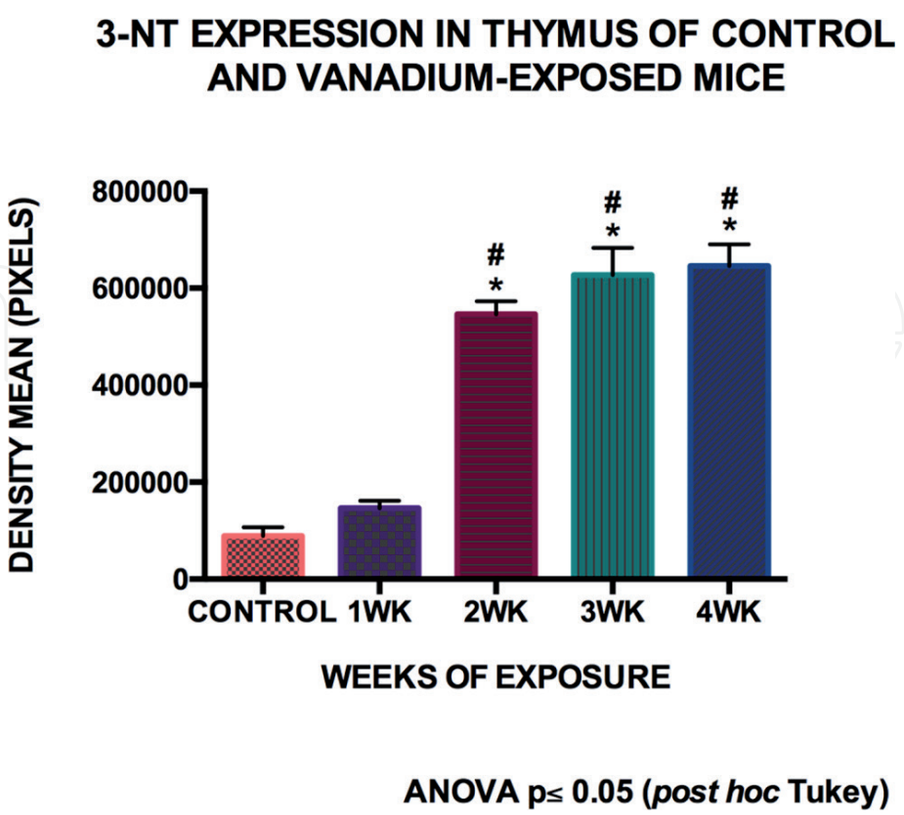

Figure 3.
Density means of 3-NT cells in the thymus (density mean ± SEM of pixels/mm²). Density values increased in V-exposed mice from week 1 to week 4. ANOVA (Tukey's) $p \leq 0.05$.

cortex/medullae ratio and by using cytokeratin-5 (K5) as a marker, the mistaken location of the K5+ cells in the medulla was demonstrated **Figure 2** [4, 30].

The density mean showed the changes in the presence of K5+, ocher-stain cells that increased during the first week of exposure compared with controls; however, the stain density at weeks 2 and 4 had a slight decrease but never reached the control values $p < 0.05$. These results showed that V produced changes in thymus cytoarchitecture, in addition of an increase in K5+ cells, changes that suggest that the T-cell selection was disrupted by V-inhalation [5]. Also, the presence of nitrosative stress as possible explanation of our findings was explored with 3-nitrotyrosine (3-NT) [38].

Our unpublished data demonstrated an increase time-dependent in the whole thymus tissue of 3-NT (**Figure 3**).

4. Conclusions

Air pollution affects the thymus. V inhalation induces nitrosative stress a decrease in DCs, the expression of CD11c and MHCII and mTEC increase, which could implicate that the negative and the positive selection were not properly completed. All these changes could allow that autoreactive clones were liberated to the blood stream and the incidence in autoimmune diseases rises in the dwellers of polluted cities [39].

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