



## Research Protocol for Control of Asthma in Children Eliminating the Inflammatory Response at the Epithelio-endothelial Level, in Upper and Smaller Airways

### Problem Submission

### Antecedents

In the past, having considered the epithelium as a passive barrier has obscured the understanding of many nosological entities and diverted genuine efforts which could have improved these diseases. This supposition has hindered the development of treatments capable of eliminating the underlying pathology. Efforts have been directed to attacking the problems, when the epithelia have been weakened and rely totally on antibacterial treatments, which can not control inflammation, and/or palliatives, which diminish symptoms temporarily, or on inappropriate anti-inflammatory therapies.

Recent discoveries have illustrated the importance of the epithelio-endothelial apparatus in starting unresolved inflammation through sustained immunitary stimulation of epithelia in asthmatic patients, like in other pathologies, where unresolved chronic inflammation is fundamentally the underlying cause. Thus these recent discoveries are shedding new light on the true nosological nature of the problem<sup>1,2</sup>. New concepts on the function of the vast epithelial and endothelial systems confirm the latter as true autocrine or paracrine organisms. These systems respond to cytotoxic signals so that they modify their behavior accordingly and, if they are excessively stimulated, their response is accompanied by pathophysiologic dysfunctions, which generate increased permeability and the weakening of barrier functions.

The above is manifested also in wound healing. Animal studies done on this subject matter in Costa Rica confirm the usefulness of eliminating such epithelial dysfunctions to bring on proper wound healing<sup>3</sup>. Completely contrasting results can be observed, in relation to the type of wound healing that can be elicited, depending on how the wound was managed. In all wounds on seven test animals where the inflammatory stimulus persisted, the process ended in ulceration with complete absence of epithelial regeneration in all seven animals. In those wounds where the inflammatory stimulus was eliminated, healing with complete epithelial orthokeratotic regeneration was observed in four of seven animals. The study comes to the conclusion that sustained epithelio-endothelial dysfunction is an obstacle for correct wound healing.

<sup>1</sup> Holtzman M. Epithelial Cells Contribute to Chronic Inflammation in Asthma. Washington University in St. Louis. Highlights of Asthma 99' Honolulu. JAMA Asthma Information Center <http://www.ama-assn.org/special/asthma/newsline/conference/asthma99/0502epit.htm>

<sup>2</sup> Devalia JL, Bayram H, Abdelaziz MM, Spasford RJ, Davies RJ. Differences between cytokine release from bronchial epithelial cells of asthmatic patients and non-asthmatic subjects: effect of exposure to diesel exhaust particles. Academic Department of Respiratory Medicine, St, Bartholomew's and The Royal London School of Medicine and Dentistry, London Chest Hospital. London UK. *Int Arch Allergy Immunol* 1999 Feb-Apr; **118** (2-4): 437-9

<sup>3</sup> Feoli E, Arrieta A, Murillo J. Regenerative wound healing with the use of NEUMACTIL, in fullthickness skin defects caused in pigs. Pre-clinical Study (Universidad Nacional Autonoma, School of Veterinary). Veterinarian Congress, celebrated at Hotel Corobici, San Jose, Costa Rica. Week of November 8, 1999.

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Several controlled studies in humans have shown preliminary results which suggest the presence of epithelio-endothelial dysfunction in large traumatic wounds and in surgical wounds<sup>4,5</sup> and in periodontal disorders<sup>6</sup>, as well. A monograph explains (see ref n°6), how the benefit of anti-inflammatory therapy can eliminate unresolved and sustained inflammation in disorders of the nasopharynx and the middle ear<sup>7</sup>.

Once the acute phase of asthma subsides, during which pre sensitized mast cells with a coating of IgE react to the same previous antigen, or cross-reacting antigens, and release histamine (causing bronchospasm); leukotrienes (attack leukocytes, eosinophils and release mucus); and platelet activating factor (which causes more release of histamine and serotonin from platelets); sound and sustained epithelio-immunitary stimuli can be identified in chronic asthma, when studying populations of T cells. Subsequently this stimulation will last indefinitely, causing delayed responses. Mounting evidence points to the fact that asthma is fostered and maintained by memory T cells, chronically activated and sensitized by allergen presentation, of occupational or viral allergenes, and which will aggregate in the lung after exposure to the aforementioned antigen or a viral infection. Allergens will provoke a response of T cells with CD4+ receptors, or helper cells Th. Virus on the other hand, will be recognized by CD8+ cells, or cytotoxic cells, described as Tc1 type. In the respiratory tract of asthmatics, the prevalence of CD4+ over CD8+ cells is the rule, so that the cytokine profile is of Th-2 and Tc-2 character. The prevalent interleukines are IL3 and IL5, granulocyte-macrophage colony stimulating factor GM-CSF and eotaxin, which mobilize and activate eosinophils that subsequently will damage the mucosa and IL4, IL3, essential cofactor in stimulating generalized production of IgE. The end result is epithelial shedding, hypersecretion of mucus and contracture of bronchial smooth muscle<sup>8,9</sup>. So that concisely, the inflammatory response seems to be dominated by the number of eosinophils in state of respiratory explosion, condition which correlates well with the severity of asthma<sup>10</sup>. The cytokine secretion of the Th2 and Tc2 type is responsible for the accumulation of eosinophils in asthma, with or without atopy, and especially with regards to the secretion of IL5 and for the final accumulation of IgE. High levels of IgE, correlate since very early ages, to chronic asthma. However neutrophils can also enter the picture and complicate the severity of asthma, increasing the oxygen radicals ORs released by the respiratory explosion of PMNs and the subsequent profibrotic changes, form liberation of proteinases, lipoperoxidases and cytokines<sup>11</sup>.

<sup>4</sup> Feoli E, Montalbert C, Bravo D, Prado Protocol for Determining Prophylaxis in Surgical Wound Infections, with the use of Polyactil July 2000

<sup>5</sup> Feoli E, Badilla V, Garcia J, Acevedo J, Gurierez. Research Protocol on the Effect of NEUMACTIL in Preventing the Infection of Exposed Fractures, Shortening and Simplification of Salvage and Reduction of Secondary Amputations. July 2000

<sup>6</sup> Feoli E. Regenerative Action of Polyactil in Periodontium. <http://www.geocities.com/NEUMACTIL>

<sup>7</sup> Feoli E, New therapeutic weapon in Otorrhinolaryngology. <http://www.geocities.com/NEUMACTIL> <sup>8</sup> Kon OM, Kay Ab. T cells and chronic asthma. Allergy and Clinical Immunology, National Heart and Lung Institute, Imperial College School of Medicine, London, UK. *Int Arch Allergy Immunol* Feb-Apr; **118**(2-4): 113-5

<sup>9</sup> Gelfand E. Cytokines in Allergic Diseases: Will the Real Culprit Please Stand Up? American Academy of Allergy, Asthma and Immunology 56th Annual Meeting Day 3 - March 6, 2000 [http://www.medscape.com/medscape/con/2000/ACAAI/Story.cfm?story\\_id=1065](http://www.medscape.com/medscape/con/2000/ACAAI/Story.cfm?story_id=1065)

<sup>10</sup> Tsai JJ, Kao MH, Hau SH. The respiratory burst activity of activated eosinophils in atopic asthmatics. Section of Allergy and Clinical Immunology, Department of Internal Medicine, Cathay General Hospital, Taipei, Taiwan. *Int Arch Allergy Immunol* 1999 May; **119**(1): 38-44

<sup>11</sup> Carrol N, Carello S, Cooke C, James A. Airway structure and inflammatory cells in fatal asthma attacks. *Eur Respir J* 1996; **9**(4): 709-715

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The endothelial involvement in inflammation of the pulmonary airways is also a clearly documented fact. Plasma tissue plasminogen activator (tPA) has long been considered to be the product of the endothelial cells that line the various parts of the vascular system regardless of vessel size or location. However Levin, Santell and Osborn, outlined experimentally the distribution of tPA in the endothelium of the mouse lung, studied by immunohistochemical analysis of normal lung tissue and it was shown that positive staining is limited to the endothelial cells of the bronchial arteries only, regardless of size, with few cells of the pulmonary circulation associated with tPA staining. The pulmonary vessels that did contain endothelial cell-derived tPA were consistently between 7 and 30  $\mu\text{m}$  in diameter. No capillary or large vessel pulmonary endothelium ever stained positive. These results were also observed in primate lung tissue where the bronchial endothelium of all vessels, even down to capillary size, contained tPA, while none of the pulmonary endothelium did so<sup>12</sup>.

The same authors demonstrated that prolonged exposure of mice to hyperoxic conditions promotes acute lung injury and associated inflammation. Using this model, the effect of inflammation on endothelial cell tPA expression was evaluated. A 4.5-fold increase in the number of pulmonary vessels staining positive for tPA was observed after 66 hours with all of these vessels having a diameter between 7 and 30  $\mu\text{m}$ . Again, none of the endothelium of large arteries or veins nor the capillaries had tPA. Whole tissue tPA mRNA increased dramatically with hyperoxia and in situ hybridization analysis showed tPA mRNA in the endothelium of the same types of vessels as antigen. The tPA localized to both the bronchial and pulmonary endothelium was active with neither tPA-PAI-1 complexes nor urokinase, inhibitors found in perfused lung tissue. These results indicate that endothelial cell tPA expression, either constitutive or induced by a pathologic event, is a function of a highly select group of endothelial cells which are defined by their association with vessels of discrete size and/or anatomic location. Thus, the widely held concept that the steady state level of plasma tPA is maintained through its constitutive production by all endothelial cells of the vascular system is invalid. Also suggested is the possibility that endothelial cell tPA might play a broader role than simply maintaining vessel patency as a component of the fibrinolytic pathway and contribute to complex dynamic processes such as inflammation.

Another important territorial association with the bronchial system is the one with the MALT (mucosa associated lymphoid tissue) system. This important system follows the bronchial distribution and reaches the bronchiolar subdivisions, and also the smaller size bronchial vessels; in the periphery more dispersed and poorly structured, however conserving the full capacity of recognizing antigen and of staging an immune response. This lymphoid tissue should be named in this territory more properly, as BALT (bronchial associated lymphoid tissue) and it is exquisitely involved in mucosal barrier immunity, which will fight pathogenic assaults; see ref.n° 7.

<sup>12</sup> Levin GE, Santell L, Osborn KG. The expression of tissue plasminogen activator in vivo: a function defined by vessel size and location. *Journal of Cell Science* 110 (2) 139-148 1997

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These findings then explain why asthma is limited to the bronchial mucosa and why patients will conserve a full ventilatory capacity (see cases below), when the inflammation afflicting this territory comes to resolution. Thus the name for the entity, of bronchial asthma, seems quite fortunate based on these findings.

The above is supported by very interesting clinical observations which demonstrate that bronchial arteries are substantially enlarged in response to chronic inflammation, which accounts for the severity of hemoptysis in patients with tuberculosis, bronchieactasis and cystic fibrosis. The association seems to correspond between inflammation most relevant in the territory where vessels exhibit tPA producing capacity. These changes are evident when studying patients with CT (computerized tomography)<sup>13</sup>. <http://rwj-rad.rwjuh.edu/newcases.d/bron3.jpg>

Even if the earlier importance attributed to neurokinins in the pathophysiology of asthma is respected: takykinins which activate substance P and neurokynin A, secreted by specific neurons that exist in the respiratory tract; their response in the end can be collectively recognized as “neurogenic inflammation”. Responses as vasodilatation, mucus secretion, plasmatic protein extravasation, leukocyte adhesion and bronchoconstriction are either allies or constituents of inflammation<sup>14</sup>.

The antecedents of this study have been only recently elucidated, establishing a revealing inflammatory nature of bronchial asthma. It wasn't until the end of 1999 that the nature of the disease in the extent of the smaller airways came to be noticed, with compromise of bronchiolar structures in the range of  $\geq 2$ mm diameter. This didn't become obvious until the necessity of developing aerosols which could deliver very small particles was appreciated. New propellants, like the hydroxyfluoroalkanes HFA, could improve the delivery of corticosteroids in very small airways, well beyond the previous figures of only a meager 5% delivery and, in theory, should change asthma radically. In spite of this well established fact, the variety of commercial preparations and the disparity of delivery in reality, throw a median figure below 60% delivery level required to diminish the regional entrapment of ventilatory air caused by the spasm of smaller airways and which is demonstrable by high resolution computerized tomography<sup>15</sup>. Leaving aside the side-effects that the long term use of corticosteroids in children (in combination or less, with antileukotrienes) might procure, it has finally been realized that the possibility of truly delivering anti-inflammatory agents at such peripheral confines seems to consist in the only possible remedy to improve the ventilatory dysfunction of asthma and to diminish the unfavorable airway remodeling and the typical diminution of lumen caliber that follows, causing the histologic changes judged by many authors as irreversible<sup>16</sup>.

<sup>13</sup> Najarian KE, Morris CS. Arterial Embolization in the Chest. *J Thoracic Imaging* 13(2): 93-104 April 1998 <http://rwj-ad.rwjuh.edu/newcases.d/bron3.jpg>

<sup>14</sup> Roisman GL, Lacronique JG, Desmasez-Dufeu N, et al. Airway responsiveness to bradykinin is related to eosinophilic inflammation in asthma. *Am J Respi Crit Care Med* 153: 381-390 1996

<sup>15</sup> Casale T. Small Airways, Large Effects. 1999 American College of Allergy, Asthma and Immunology Annual Meeting November 2-13.

[http://www.medscape.com/medscape/con/1999/ACAAI/Story.cfm?story\\_id=882](http://www.medscape.com/medscape/con/1999/ACAAI/Story.cfm?story_id=882)

<sup>16</sup> Panettieri RA. Airway remodeling: the therapeutic approaches. Program and abstracts of the 1999 Annual Meeting of the American College of Allergy, Asthma, and Immunology; November 12-17, 1999; Chicago, Ill. Plenary: Asthma as irreversible lung disease.

[http://www.medscape.com/medscape/con/1999/ACAAI/Story.cfm?story\\_id=893](http://www.medscape.com/medscape/con/1999/ACAAI/Story.cfm?story_id=893)

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## Problem definition

Statistics that show an increase in the prevalence of asthma in the fully industrialized world, since 1980 and onwards, at a rate of annual growth of 5%<sup>17,18</sup> are indicative of therapeutic measures that do not modify the etiopathogenesis of the disease, with no apparent indication of what is causing the increase to clinicians managing the disorder. Notwithstanding, it is important to emphasize the possible worsening of the disease from environmental causes, through the atmospheric contaminants and the effects that combustion gases can arouse in the epithelial cells of the respiratory system (see ref. n° 2). There is conclusive evidence that these cells are hypersensitive to such contaminants; either by exposure to particulate diesel emissions or high ozone levels of inhaled air. It is quite cumbersome to concede presence of chronic illness in children and that asthma, without any doubt the most frequent ailment in early age, has been on the rise for well over two decades.

Since the depositing of corticosteroids in the finer airways is increasingly being considered as the only weapon that can fight asthma and its evolution, by some degree of resolution of its inflammatory etiopathogenesis, it is still unknown what adverse effects might arise from the delivery of higher doses in more extended areas of the respiratory epithelium, when treatment must be prolonged. It is quite probable that a higher efficiency of delivery will increase the systemic absorption of the steroids; therefore it would be reasonable to ask ourselves: What impact will the new therapeutic efficiency have on children's general health and their overall well-being? Systemic effects of corticosteroids administered by inhalation are dependent upon the extensiveness of drug deposited directly onto the respiratory epithelium. With less efficient methods, 15 to 30% ends up on the respiratory surface and 70 to 85% is eventually swallowed with material ending up in the oropharynx or nasopharynx. To some degree, the ingested drug can be rendered inactive at the gut level. The rest of the drug is absorbed and successively metabolized reaching its first pass through the liver. The drug escaping liver metabolism, and 100% of drug absorbed at the respiratory epithelium gaining access into the pulmonary circulation, can cause unfavorable systemic effects. More finely particulated aerosols, aiming at increasing topical delivery, will very likely succeed in tossing more corticosteroids into the system. Topical corticosteroids delivery is potentially more damaging than oral administration. Three separate studies on the response to different doses of budesonide and fluticasone, taking into account corrections for topical efficiency, show a comparative 1.5 fold bioactivity systemic increase, when the finely sensitive parameter of suprarenal suppression is measured.

Fluticasone propionate, exhibited suppressor activity of 2.5 fold increase and in a meta-analysis

<sup>17</sup> Measuring Childhood Asthma Prevalence Before and After 1997 Redesign of the National Health Interview Survey – United States *Morbidity and Mortality Weekly Report* 49(40): 908-911, 2000

<sup>18</sup> Martinez FD. Evolution of asthma through childhood. Program and abstracts of the 1999 Annual Meeting of the American College of Allergy, Asthma, and Immunology; November 12-17, 1999; Chicago, Ill. Plenary: Asthma at the extremes of life.

[http://www.medscape.com/medscape/con/1999/ACAAI/Story.cfm?story\\_id=888](http://www.medscape.com/medscape/con/1999/ACAAI/Story.cfm?story_id=888)

<sup>19</sup> Clark DJ, Lipworth BJ. Adrenal suppression with chronic dosing of fluticasone propionate compared with budesonide in adult asthmatic patients. *Thorax* 1997; 52: 55-58

<sup>20</sup> Boorsma M, Andersson N, Larsson P, Ullman A. Assessment of the relative systemic potency of inhaled fluticasone and budesonide. *Eur Respir J* 1996; 9: 1427-1432

<sup>21</sup> Donnelly R, Williams KM, Baker B, Badcock CA, Day RO, Seale PJ. Effects of budesonide and fluticasone on 24 hour plasma cortisol: a dose response study. *Am J Respir Crit Care Med* 1997; 156: 1746-1751

<sup>22</sup> Lipworth BJ, Wilson AM. Dose response to inhaled corticosteroids: benefits and risks. In: Chapman K, ed. *Semin Respir Crit Care Med*, 1998;19:633-54.

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of 22 studies, becometasone dipropionate exhibited a 2.1 fold increase and triamcinilone acetonide, a 3.6 fold increase<sup>19,20,21,22</sup>. (Centers for Disease Control)

Dysfunctions related to the altered hypothalamic/pituitary axis involve growth inhibition, glaucoma and cataracts, osteoporosis and skin thinning which, taken aside from the respiratory impairment, reduce quality of life increasingly<sup>23</sup> and are closely related to corticosteroid utilization. Finally a consequence arising from the direct application of steroids, it is represented by the danger of developing oral candidiasis.

In view of the above, it is reasonable to formulate the following question: Are we fighting asthma from its ethiopathogenetic perspective? The problem, in spite of this ethiopathogenesis of asthma having been defined scientifically, is that we lack the proper weapons to control this basic pathology. The other problem resides, before other adequate therapies are mentioned, in the fact that judging from available statistics and very recent experimental results, serious faults can be identified with accepted management of the disease, which relate strictly to the inability to solve asthma from the ethiopathogenetic perspective.

### **Justification of study**

The best justification for this research stems from the same researchers that have explored the inflammatory aspects of the disease and are continuing with the quest. The great majority is asking for the urgent development of anti-inflammatory weapons that can quench the tenacious stimuli that perpetuate the disorder and which, according to some, become irreversible and culminate in a continuum, with origin in infancy and ending with asthma of adults. Researchers center their hopes in anti-inflammatory therapy, as the best likelihood of truly improving the ailment.

More direct and circumstantial evidence, does justify looking at new therapeutic possibilities, as is the use of NEUMACTIL, due to its specific properties in placating the inflammatory process of asthma and the resulting permanent steadfast improvement observed, without incurring risks. The product's favorable toxicity profile<sup>24</sup> and the simplicity of delivering the active principles by topical application are quite attractive for adopting the method diffusely and with possibility of benefiting large populations. In a series of 30 patients from ages ranging form 2 to 24 years, it has become possible to eliminate symptoms completely, starting from the first day of treatment and experiencing an ensuing gradual improvement, until, in a very short time,

<sup>23</sup>Lipworth B. Systemic adverse effects of inhaled corticosteroid therapy: a systematic review and metaanalysis. *Arch Intern Med.* 1999; **159**:941-955.

<sup>24</sup> Feoli E, Ramirez JJ, Chavez MA. Acute and subchronic oral toxicity of NEUMACTIL in mice. Unidad de Ensayos Biologicos, Universidad de Costa Rica Nov 2000

<sup>25</sup>Ramirez JJ, Feoli E. Casuistica de 30 casos de ninos asmaticos tratados con NEUMACTIL. Communication personal. [info@medactil.com](mailto:info@medactil.com)

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children are reinstated to a normal life without restrictions<sup>25</sup>. Elimination of asthmatic symptoms and prevention of later consequences due to unresolved inflammation, plus reinstatement of these children to a quality of life with nothing to envy from the normal population, with any doubt, justifies the study.

### Case evidence

Name	Locality	Current Age	Age at Asthma Initiation	Previous Condition	Improvement Start	Time to Stable Condition	Stable	Other Treatment	Post Physical Activity	New Crisis
Marytere Ramirez Monge	Heredia	10	1	Physical restrain	1 ½ hrs	Stable at 6 days	3 months	Antihistaminic Expectorant	Physical activity +++	None
Miguel A. Rojas	San Jose	8	1	Physical restrain	1 ½ hrs	Stable since	1 month	No other treatments	Physical activity +++	None
Mauricio Madrigal	Heredia	12	8	Physical restrain	4 days	Stable since	2 months	No other treatments	Physical activity +++	None
Natasha Yauger	Moravia	5	Birth	Physical restrain	4 days	Stable since	2 months	No other treatments	Physical activity +++	None
Enrique Aguilar	San Jose	10	1	Physical moderate	2 days	Stable at 5 days	1 month	No other treatments	Physical activity +++	None
Jose Daniel Ramirez	San Jose	4	1	Prev hospitalization	4 days	Stable at 5 days	2 ½ months	Expectorant	Regained activity	None
Ernesto Guevara	San Jose	3	2	?	1 ½ hrs	Stable at 5 days	3 months	No other treatments	Active child	None
J.R.	Ciudad Colon	5	4	Physical moderate	1 ½ hrs	Stable since	3 months	No other treatments	Physical activity ?	None
T.R.	Ciudad Colon	5	4	Physical moderate	1 ½ hrs	Stable since	3 months	No other treatments	Physical activity ?	None
Diana Arino	San Jose	13	3	Physically moderate	2 days	Stable since	2 months	No other treatments	Physical activity ?	None
Maria Mercedes Cruz	San Jose	24	Birth	Physically moderate	4 days	Stable since	1 month	No other treatments	Physical activity ++	
Miguel Chavarria CF	San Jose	4	Birth	Physically crippled	1 <sup>st</sup> day	Stable since	2 months	Yes, unknown treatment	Physical activity notably improved	None
Miguel Rojas Chapata	San Jose	8	Birth	Serious previous hospitalization	5 days	Stable since	1 month	Expectorant	Regaining physical activity	None
Yerich Gonzalez	Heredia	12	6	Unable to exercise	2 days	Stable since	1 ½ months	Expectorant	Physical activity +++	None
Lena Espinoza	San Jose	18		Moderate exercise	1 day	Stable since	1 month	No other treatments	Physical activity +++	None
Valeria Porras	San Jose	5	Birth	Moderate exercise 1 ½ hrs	1 day	Stable since	1 month	No other treatments	Physical activity +++	None
Fabian Porras	San Jose	3	Birth	Moderate exercise 1 ½ hrs	1 day	Stable since	1 month	No other treatments	Physical activity +++	None
Fernando Porras	San Jose	1	Birth	1 day	1 ½ hrs	Stable since	1 month	No other treatments		None
Carlos Brenes Solano	San Rafael de Oreamuno	12	2	Unable to exercise	Favorable crisis after starting MH (no hosp	→ stable cond	2 months	No other treatments	Physical activity +++	None

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					yields to MH)					
Melany Soto Orozco	San Jose	3	1	Previous hospitalization	2 hrs	Stable since	1 month	No other treatments	Regaining physical activity	None
Josue Hernandez Barrantes	Tibas	4	1	Physical moderate	1 ½ hrs	Stable at 3 days	1 week			N. A.
Joseline Hernandez Barrantes	Tibas	1	Birth		1 ½ hrs	Stable at 3 days	1 week			N.A.
Maria Vargas Cruz	Alajuelita	3	1	Physical moderate	4 days	Stable at 5 days	1 month	Expectorant	Physical activity ++	None
Katia De Bendictis	San Jose	24	Birth	Physical moderate	4 days	Stable at 5 days	15 days	No other treatments	Physical activity +++	None
Pamela Madrigal	San Jose	3	Birth	Physical moderate	2 days	Stable since	15 days	No other treatments	Physical activity +++	None
Daniela Ramirez	San Jose	6	Birth	Physical moderate	2 days	Stable since	15 days	No other treatments	Physical activity +++	None

Notes: 5 children have had colds during stability and didn't develop bronchospasm and didn't require medication other than expectorants. One of the two had allergic response to milk and even though she had histamine release, didn't develop bronchospasm.

Patient Miguel Chavarria is a Cystic Fibrosis patient.

3 patients had recent previous hospitalization

## Objectives

### General

To study the efficacy and tolerance of NEUMACTIL, in treating a group of children with asthma, from medium to severe in intensity, and to introduce this new method so that it can be affirmed as a specific treatment of the disease.

### Specific

To quantitatively define the efficacy of NEUMACTIL in eliminating the clinical manifestations of asthma in children in the study group and to substantiate return to physical activity performance, without restrictions. In terms of inflammatory markers: To define the abatement of the ethiopathogenetic inflammatory process and to quantify the improvement of the ventilatory capacity of the study group.

### Hypothesis

Circumstantial evidence of children treated with NEUMACTIL and returning to a lifestyle without physical restrictions attest of the study putting to test the cause-effect relationship

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between use of NEUMACTIL and the huge benefits observed. However, since these benefits stem from an astounding simple method, it is opportune to define how the product is delivered and how its action will influence favorably the smaller airways. As cited previously the product, in the form of a gel, is rubbed-in onto the skin of the posterior chest twice daily: in the morning and before bedtime. This evidence implies that it is possible to deliver the product transdermally, and within the span of 1 to 2 hours, reaches the bronchiolar environment, where it will manifest its therapeutic action in the epithelio-endothelial apparatus. Thus delivery through the bronchial arteries seems to be achievable; which would be quite revealing: for inside of the walls of the tracheobronchial tree are embodied the tissues affected by the inflammatory disorder and eventually irrigated by these arteries. This explains the marked specificity of this treatment in controlling the symptoms, most probably due to high containment of delivery through dilated bronchial arteries (that can be easily catheterized) and reaching a specifically involved anatomic location. The delay of 1 to 2 hrs in order to start effect is explained by the route that the active principle must follow in order to reach the left chambers of the heart and the aorta, and while it reaches the concentration that would initiate therapeutic action. The proximity of the chest wall to the venous return reaching the right heart confirms the convenience of the product's application in the elected area. The aortic origin of the bronchial arteries between the fourth and sixth vertebral bodies most likely facilitates relatively early access of the product to its target territory. Finally since it has been observed that the bedtime application protects the patient through the extent of the night, its action necessarily must belong to a retarded and controlled category.

This study is structured under the hypothesis that a population sample of children with asthma, observed experimentally while treated with NEUMACTIL, will show prompt alleviation of symptoms and will improve markedly its ventilatory condition after 2 to 3 days. These benefits supervene after trans-dermal delivery of NEUMACTIL in its gel form and represent, from an ethiopathogenetic standpoint, control of inflammation at the bronchiolar epithelium, due to the striking anti-inflammatory therapeutic activity of the product.

### **Method and materials**

This study as it was indicated, it is intended as an experimental one, in order to determine in the study group, a direct cause-effect relationship between a variable and the described benefits.

Population, sample, selection criteria for inclusion in the sample and definition of variables:

A population comprising 50 children from 7 years to 18 years, diagnosed by specialists to be afflicted by asthma, of at least 1 year duration and who are receiving treatment with B2

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agonists and/or inhaled corticosteroids and/or antileukotrienes. A few individuals older than 18 and up to 25 can be included in the group. The Costa Rican study will be with volunteers and to be carried out in private facilities. However studies done in other countries can be carried out in public facilities, depending on the availability of patients to researchers. The individuals or their parents will sign an informed consent. For ethical reasons however, the study group will not be compared to a placebo group, considering the very high efficacy and constancy of results observed preliminarily, on patients of the non-controlled series. Individuals will be evaluated previously by a pediatrician neurologist and a baseline FIVE taken and also non-invasive inflammatory markers that are specified below, where dependent variables are defined. The independent variable consists in treating patients with NEUMACTIL. The dependent variables are the clinical benefits perceived and the improvement in FIVE and the observed decrease in levels of non-invasive inflammatory markers.

### **Method**

Whoever is responsible for the patient, or adult patients themselves, will sign the informed consent. See annex. Since the study is with volunteers, relatives of the patient will have acquired a good deal of knowledge of the study and be familiarized at the time when consent is presented for signature.

### Data collection

Data will be gathered by registry and a small record for medical keeping will be available at the medical facility where individuals will be studied. The Costa Rican record is shown as an annex. Each team can draw its own medical record.

### FEV<sub>1</sub>

This common test is recognized worldwide as a faithful representation of ventilator status of asthmatic individuals.

### Non-invasive inflammatory markers

These are objective criteria of the inflammatory state at the epithelio-endothelial apparatus of respiratory system, which are significantly elevated in asthmatic children. The improvement of levels of inflammatory markers is to be considered indicative of favorable response to therapy.

### Exhaled NO

Endogenous nitric oxide derives from the amino acid L-arginine through action of NO synthases. There are three synthases –neuronal and endothelial– plus the third of epithelial

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origin, which is inducible, denominated ion (nitric oxide inducible synthases). The expression of ion in respiratory epithelial cells increases under the influence of pro-inflammatory cytokines, IL-1 and TEN types. The inducible synthase will provoke NO production, at 1000 times more intensity than the constitutive ones. The vasodilatation that NO will bring about increases the flux in post-capillary venules and is contributive to inflammation through the edema that will prevail in the respiratory tract. The vascular relaxation is due to neuronal stimulation (see above about 26 Broide DH. Lower Respiratory Tract Inflammation: Noninvasive Markers. 96th International Conference of the American Thoracic Society [http://www.Medscape.com/medscape.com/2000/ATS/Story.cfm?story\\_id=1245](http://www.Medscape.com/medscape.com/2000/ATS/Story.cfm?story_id=1245) neurogenic inflammation). This situation will unbalance the equilibrium between ventilation-perfusion, causing an increase of mucus production of the submucosal glands.

Elevation of NO will favor the Th2 profile in lymphocytes, fact that will protract the asthmatic response. The source of exhaled NO, originates from the action of iNO synthase. This can be observed by immunohistochemical studies, in biopsies of respiratory epithelium of asthmatics where special stains, can mark specifically the iNOs. Activated macrophages in a state of respiratory explosion can also contribute to the levels of exhaled NO. The direct bioactivity of NO properly as a molecule is unclear. On the one hand it increases chemotaxis of neutrophils, monocytes and eosinophils trough a GMP (guanosine monophosphatase) dependent mechanism, but on the other, it is inhibitory for the adherence of leukocytes to vascular and bronchial endothelia<sup>27</sup>.

The concentration of NO in exhaled air is measured with chemoluminescence. In atopic children with a median age of 11.1 ± 0.8 years, values of 19.4 ± 3.3 ppb can be expected, compared to 4.0 ± 0.5 ppb in normal children. The levels of NO, correlate with the absolute quantity of eosinophils in the blood<sup>28</sup>. Information on method is provided, enclosed.

Eosinophils and PCE, or eosinophilic cationic protein, in the sputum

Both parameters analyzed in the sputum induced by inhalation of hypertonic saline give very good correlation among themselves. The first is carried out by analyzing separate plugs which are selected and taken from the specimen, with an equal volume of dithiothreitol 0.1%, or “cytopins”, in order to carry out the counts, and a special stain and separation of supernatant, for PCE. The latter is determined by fluoroimmunoassay. Jang and Choi, have correlated the

<sup>27</sup>Volcheck GW, Li JTC. Mechanisms of Asthma: The Roles of Neurokinins, Nitric oxide and Genetics. Medical School, Division of Allergy and Outpatient Infectious Disease and Internal Medicine, Mayo Medical Center, Rochester, Minn. *Medscape Respiratory Care* 3(1), 1999 <http://www.medscape.com/Medscape/RespiratoryCare/journal/1999/v03.n01/mrc.4638.volc/pnt->

<sup>28</sup>Silvestri M, Spallarossa V, Yourukova F, Battistini E, Fregonese B, Rossi GA. Orally exhaled nitric oxide levels are related to the degree of blood eosinophilia in atopic children with mild-intermittent asthma. *Eur Respir J* 1999; 13:321-326

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count of eosinophils and levels of PCE in the sputum with the disturbances of FEV1<sup>29</sup>. Levels to be expected of PCE in the sputum are: 1117.8 [2] 213.9 µgm in comparison to 154.6 [2] 47.4 µgm, in normal individuals. The convenience of determining PCE is that it will persist elevated almost for 3 days after an acute attack and has high value in differentiating recrudescences. To induce the sputum in children, it is advisable to work only with children 7 years or older.

#### Exhaled breath condensate

Patients breathe through a mouth piece into Teflon tubing, which transports exhaled breath into a container of dry ice. Approximately 1 mL of exhaled breath condensate collects in the Teflon tubing after a 5-minute period of breathing. The acellular frozen breath condensate can later be analyzed for mediators. In limited studies in COPD and asthma, H<sub>2</sub>O<sub>2</sub> and cytokines (interleukin-1 and tumor necrosis factor) have been measured. A recent study from the University of Virginia used a similar technique to demonstrate that the pH of the exhaled breath condensate in asthmatics decreases from 7.4 prior to an acute attack of asthma to 5.5 during an attack of asthma, and then returns to a normal pH of 7.4 as therapy induces a resolution of the attack of asthma. The cause of the acidification of the airway as well as any functional effects of the acid pH on airway cellular function is at present unknown.

A first visit will have the purpose of selecting candidates to be included in the study group. All selected individuals will be subjected to baseline test specified in methods. A second visit will be scheduled at 15 days, to follow the improvement of the individuals, and a final one at 30 days, when the tests will be repeated.

#### Instructions for use

Once baseline tests are completed, treatment with NEUMACTIL will be commenced. NEUMACTIL gel contains 1.4mg of phosphazene in 1gm of gel. Apply 1.4 mg of phosphazene or 1gm in the posterior chest, on skin prepared with a brief massage. After the gel is applied onto the skin, rub until the hand advances with difficulty. Application can be extended a bit towards the posterior neck. Carry out the procedure twice daily, in the morning after bathing and before bedtime. During an asthma attack, application can be extended to the anterior chest and dose duplicated to 2,8 mg of phosphazene .

<sup>29</sup> Jang AS, Choi IS. Eosinophil activation markers in induced sputum in asthmatics. Department of Internal medicine, Chonnam University Medical School, Chonnam University, Kwangju, Korea. *Korean J Intern Med* 2000 Jan; **15**(1): 1-7

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Caution: The first and perhaps the second application of NEUMACTIL might cause mild blushing, due to histamine release. However in all cases this reaction has been observed to be benign and lacking manifestation of more serious vasoactive responses. After accumulated histamine is exhausted, patients do admirably well when receiving their medication. This is remarkable! It seems to uphold the far-reaching action of trans-dermal treatment, within the bronchial artery territory. None of the patients was administered antihistamines, when receiving their medication with NEUMACTIL.

#### Nasal treatment

After having rubbed externally a small quantity of gel, use the grab end of a sterile applicator, to introduce gently sufficient gel in both nasal orifices and apply to nasal walls. This will clear obstruction of nose, making it possible for these children to breathe humid air and to inhale particles of right size that were adequately screened.

#### Statistical Analysis

Once data is gathered, statistical extrapolations will be feasible in order to determine the degree of asthma diminution, in comparison to the prevalence data of each geographic region. Analysis of data will make possible to reach conclusions on the general health and quality of life improvement for these children and the diminished social costs of the disorder.

#### **Informed consent**

According to the ethical committee requirements, for each region.

#### **Data gathering record**

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