Neurotensin Localization in Adenomatoid Cystic Malformation Versus Normal Lung: Preliminary Report of Six Consecutive Cases

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● **Neuropeptides are considered a new class of neurotransmitters, several of which interact with the immune system as well as the macrophagic activity. Among these, neurotensin (NT) enhances the phagocitic response of macrophages and is the only neuropeptide that can enhance the cytolytic effects of activated macrophages. In this way, it may play a role as an inflammatory mediator. In order to investigate the possible relationship between NT and the defence mechanisms of the lung, we started to localize the presence of NT in pulmonary adenomatoid cystic malformation (CCAM). This series consists of 6 children affected by CCAM. In every case, at operation, we obtained specimens of both normal and pathological lung. Tissue sections from the pathological lung showed a significant increase of NT-like immunoreactivity in respect to sections of normal lung. NT influences and activates the macrophages, thus suggesting that it could represent a defence mechanism in children's lung activated in some malformative conditions. Finally, the increasing evidence of NT immunoreactivity in CCAM could explicate an in utero infectious pathogenesis of this malformation.**

INDEX WORDS: Congenital cystic adenomatoid malformation, neurotensin.

INCREASING evidence has been accumulated in recent years that indicates that the neuroendocrine system can affect the immune function. The effects of neuroendocrine peptide hormones in the immune system suggest that, in addition to their classical neuroendocrine actions, neuropeptides explicate an immunoregulatory role. Recent results have shown that immunocompetent cells can produce neuroendocrine peptides and that receptors for neuroendocrine hormones are present on such cells.[1] These considerations provide the molecular basis for interactions between neuroendocrine and the immune system.

Neuropeptides are actually considered a new class of neurotransmitters, several of which interact with the immune system as well as with macrophagic activity. Among these, neurotensin (NT) enhances the phagocitic response of macrophages and is the only neuropeptide that can enhance the cytolytic effects of activated macrophages; in this way, it may play a role as an inflammatory mediator.[2]

In order to investigate the possible relationship between NT and the defence mechanisms in lung bud anomalies, we aimed to localize the presence of NT in congenital cystic adenomatoid malformation of the lung (CCAM).

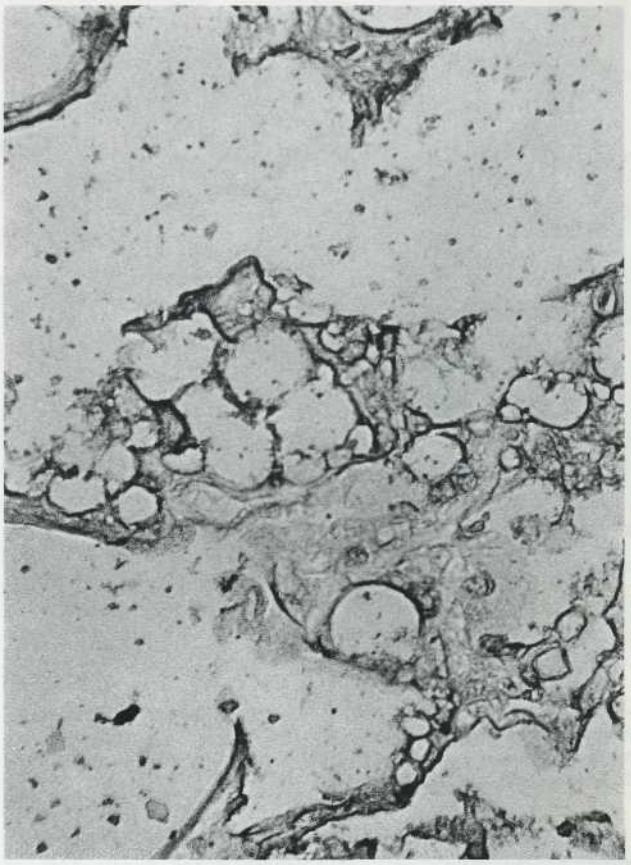

Fig 1. Photomicrograph of a tissue section of a lung affected by CCAM. Immunoperoxidase. Antibodies, anti-NT. Note the NT-like immunoreactivity, in particular localized around microcystic spaces (grey-colored tracts). (Original magnification ×250.)

MATERIALS AND METHODS

This series consists of six children affected by CCAM; there were four girls and two boys ranging in age from 1 day to 8 years (mean, 31 months; three patients aged less than 1 month at operation). In three patients the diagnosis was made antenatally by ultrasound: no maternal hydramnios or fetal anasarca were detected and all these children remained asymptomatic at birth.

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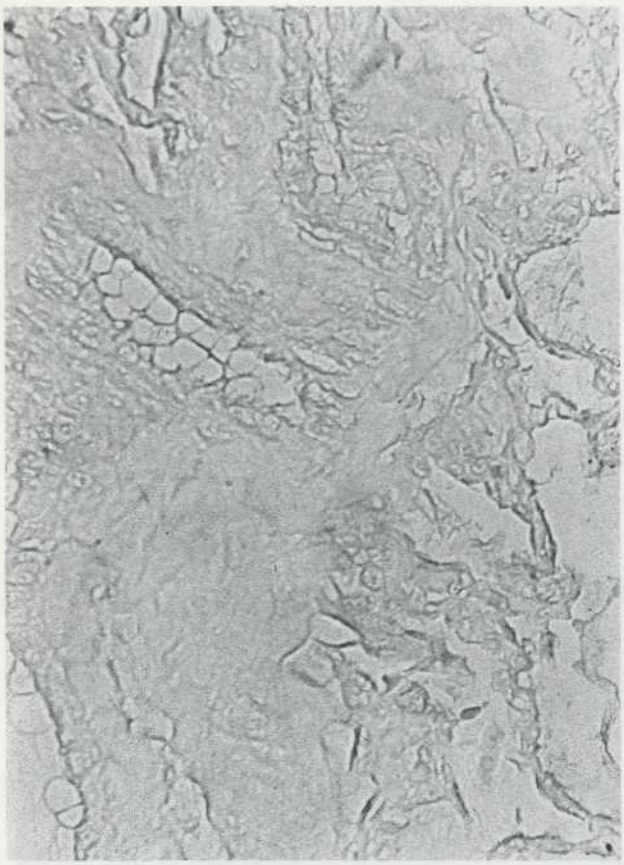

Fig 2. Photomicrograph of a tissue section of a normal neonatal lung. Immunoperoxidase. Antibodies, anti-NT. NT-like immunoreactivity is very weak, nearly absent. Note the difference in respect to CCAM (original magnification ×250).

In all but one a lobectomy was performed, whereas a left superior trisegmentectomy was accomplished in the last one. There were four cases of type II and two of type I following the Stoker classification.[3]

We obtained specimens of both normal and pathological lung in every patient at the operation. Samples of 5 to 6 × 7 to 8 × 7 to 8 mm were fixed in Bouin's fluid (picric acid 15 mL + formalin 5 mL + glacial acetic acid 1 mL) for 36 to 48 hours, embedded in paraffin, and cut by the use of an Automicrotome Jung 2050 (Germany). Sections were then tested by policlonal antibodies anti-NT, diluted in phosphate-buffered saline (PBS) BSA (1% albumin diluted in PBS), by the Streptavidine-biotin-peroxidase technique.

In detail, sections were dehydrated through an ethanol series with decreasing concentrations. In order to exclude any background due to endogen peroxidase, two adjunctive passages were performed in methanol + H_2O_2 0.5% and ethanol + HCl 0.2%. After washing the sections in PBS and PBS-Triton 0.3%, slides were covered with the first antibody (anti-NT) in a wet chamber for 1 hour. Then the sections were washed in PBS and PBS-Triton and covered with the second antibody (Rabbit Ig biotinylated whole antibody), diluted in PBS-BSA 1:200, in a wet chamber for 1 hour. After a new washing in PBS and PBS-Triton, the sections were covered with the Streptavidin-biotinylated horseradish peroxidase complex, diluted in PBS-BSA 1:400, for 15 minutes. Slides were washed again in PBS and PBS-Triton, then covered with 3,3′-diaminobenzidine activated with H_2O_2 30 volumes, diluted in PBS, for 15 minutes in a wet chamber. After a final washing, sections were passed through a series of ethanol with increasing concentrations and finally mounted with Eukitt. Control sections were covered with PBS-BSA alone instead than first antibody. The observations were performed using a Zeiss photomicroscope (Jena, Germany).

RESULTS

Tissue sections of pathological lung showed a significant increase of NT-like immunoreactivity with respect to sections of normal lung samples of the same patients. Immunoreactivity for NT was localized around multiple and irregular bronchiolar-like cystic structures and at the level of thin delicate membranes separating these abnormal small cystic spaces ("pseudoalveolar septa") (Fig 1). Tissue sections of normal lung showed a weak, nearly absent NT-like immunoreactivity (Fig 2).

DISCUSSION

CCAM is characterized by the presence of abnormal bronchiolar structures of varying sizes and/or distribution and is considered a relatively common hamartomatous lesion of unknown origin (Figs 3 and 4).

Fig 3. Photomicrograph of a tissue section of a lung affected by CCAM (H&E, original magnification ×250).

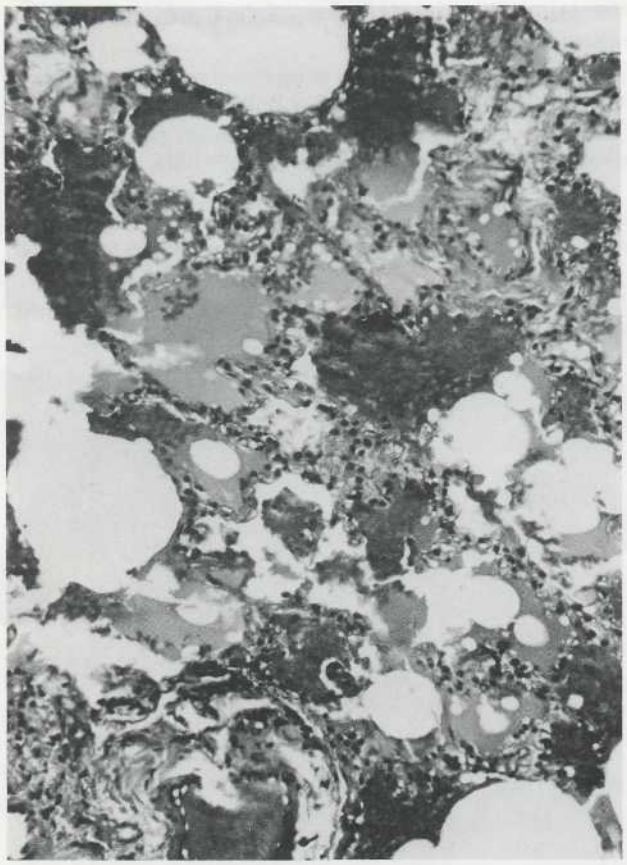

Fig 4. Photomicrograph of a tissue section of a normal neonatal lung (H&E, original magnification ×250).

The embryonic insult resulting in CCAM would appear to occur at the time when lobar architecture has been established and before cartilaginous anlage are formed (5th to 6th week), resulting from cessation of bronchial maturation and concomitant increasing of mesenchimal elements, which produces the adenomatoid appearance of the anomaly.[4]

Although the basic composition of the mass appears histologically to be an overgrowth of distal bronchiolar tissue, no proof of such hypothesis is available.[5]

The clinical picture of CCAM varies with the age at presentation, often requiring urgent treatment in the neonatal period for the rapidly progressive respiratory distress syndrome, while in older infants often assumes a chronic course characterized by recurrent respiratory infections and failure to thrive or is completely silent and is detected during a routine examination of the thorax for other reasons (Table 1).[6,7]

Table 1. Pathogenic Event and Different Evolutions of CCAM

Intrauterine infection
Evolution in CCAM
In utero resolution
Partial
Total
In utero exitus
Postnatal evidence of CCAM (NT Defence mechanism)
Different clinical aspects at various ages
Neonates
Early neonatal exitus
Respiratory insufficiency
Asymptomatic
Children
Recurrent respiratory infections
Adults
Occasional report

Whatever the clinical picture, this malformation always requires surgical therapy, and lobectomy or segmentectomy is the treatment of choice.[8]

The role of NT is better known in the central nervous system rather than in other organs.[9] Possible effects of NT on gastrointestinal and pancreatic secretions and a role in arterial pressure regulation in humans have been observed and discussed in recent years.[10-12]

In 1989, Moore et al described the role of NT as an inflammatory mediator, describing both its direct effects on mature phagocytic leukocytes stimulation and its in vitro influence on new mononuclear phagocyte production.[2] This peculiar NT activity as an immunomodulator suggests to us a new hypothesis of pathogenesis regarding CCAM. In fact, the presence of large perialveolar collections of NT immunoreactivity in CCAM and its absence in normal lung tissue, could support an infectious in utero pathogenesis of this abnormality (Table 1); in addition, this explanation would support some described cases of partial or near-total to total spontaneous in utero resolution of CCAM.[13]

These results demonstrate an increase of NT-like immunoreactivity in tissue sections of the lung affected by the CCAM. Because NT influences and activates the macrophages, we suggest that NT could represent a defense mechanism in children's lung activated in some malformative conditions and, finally, a foretoken of the first pathogenic event.

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