MICROBIOLOGY AND IMMUNOLOGY

Specific Features of the Candidal Infectious Process under Conditions of Polychemotherapy

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Foci of candidal pneumonia in the presence of iatrogenic immunodeficiency and dysbacteriosis caused by preinjection of cyclophosphamide and ampiox were examined by histoautoradiography and light and electron microscopy. Not only did the pathomorphogenesis of *Candida* infection, type of manifestation of tissue defense reactions, and degree of manifestation of the pathogenic characteristics of the agent change under such conditions, but the mediation of the inflammation as well.

Key Words: *immunodeficiency; pulmonary candidiasis; morphology; drug treatment*

The incidence of *Candida* carriership and the incidence of candidiasis are steadily increasing [7,9, 10,15], a contributing factor being the wide prevalence of immunodeficiency states [1-3]. Clinical studies of *Candida* infection are difficult because they involve differentiating among the pathomorphological changes caused by the underlying disease, by the drugs administered, and by the *Candida* infection proper [4]. In experimental candidiasis either only the host organism is exposed in order to induce an immunodepressive state and activate the fungal microflora [13,14] or dysbacteriosis is induced in order to eliminate bacterial competition [6]. Clinically, however, an infectious process most frequently develops under conditions of combined drug treatment [12]. Just a few publications have been devoted to this topic [11].

This study was aimed at investigating the pathomorphogenesis of experimental candidal pneumonia developing during combined treatment with a cytostatic and antibiotic in the presence of induced immunodepression and dysbacteriosis.

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MATERIALS AND METHODS

A total of 387 male guinea pigs weighing 230 to 270 g were used in the experiments. Under light ether narcosis the animals were intranasally infected with a suspension of *Candida albicans* in isotonic NaCl in a dose of 1×10^6 fungal corpuscles per animal. The animals were sacrificed by ether overdosage 3 h to 30 days postinoculation. The lungs, parenchymatous organs, and organs of immunogenesis, such as bone marrow, thymus, spleen, and paratracheal, bifurcation, mediastinal, and mesenteric lymph nodes were examined. Histological and cytological specimens were prepared and histoautoradiographic and electron microscopic studies carried out routinely [5,8].

The immunocompetent system was assessed in accordance with WHO recommendations.

RESULTS

Infection of animals with a fungal culture with their immune system intact was the first stage of the study. Pneumonia developing in guinea pigs in response to inoculation with a *Candida albicans* cul-

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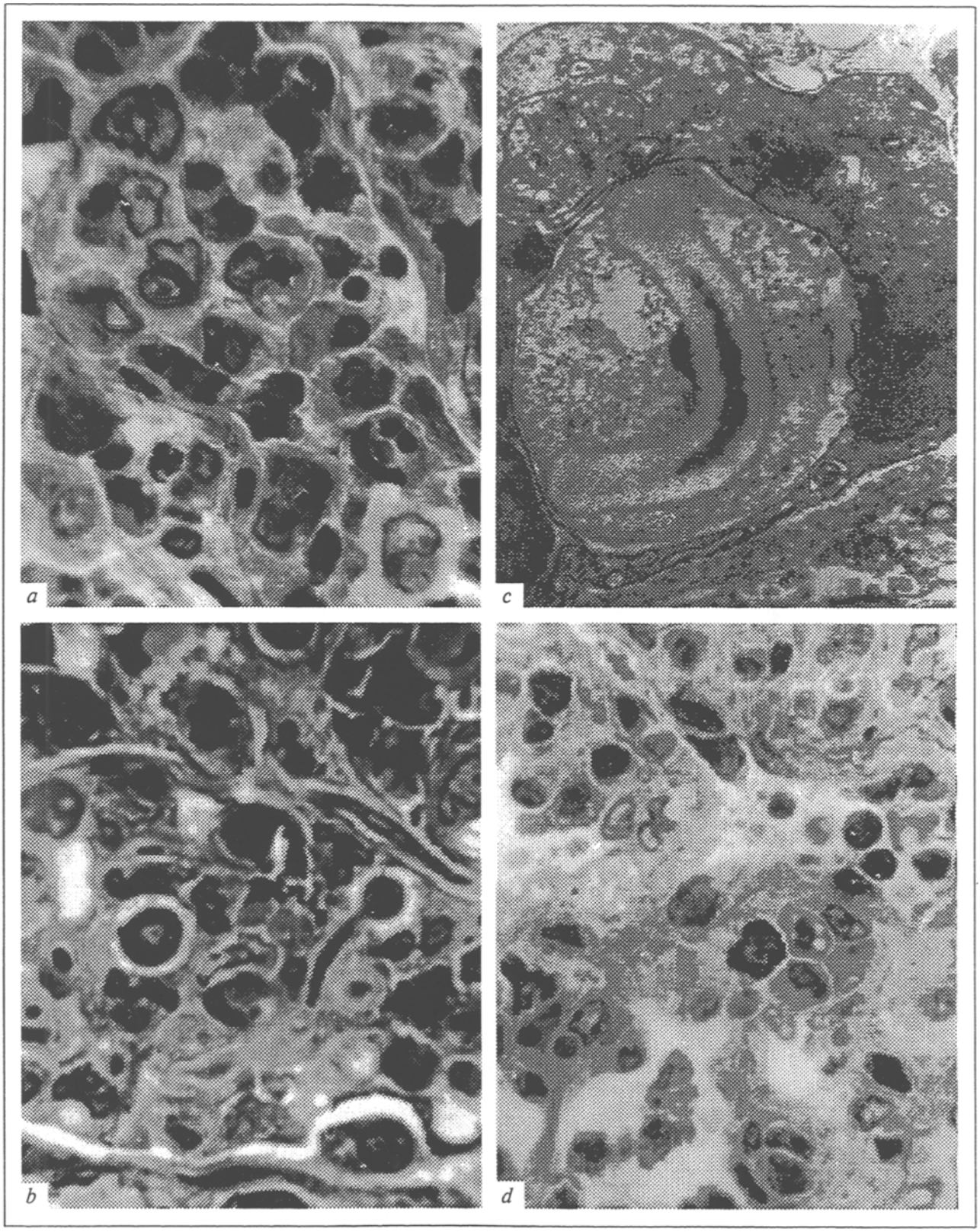

Fig. 1. Morphological picture of a focus of *Candida*—induced pneumonia under conditions of intact resistance. *a*) 24 h postinoculation. Alveolar lumens are densely lined with leukocytic—macrophagal exudate, active phagocytosis of fungal blastospores; *b*) formation of growth tube in a focus of pneumonia; *c*) collapsed fungal cell wall in phagocyte cytoplasm. Electronogram, ×140,000; *d*) mast cells in alveolar lumen. *a*, *b*, *d*: semithin slices. Methylene blue—basic fuchsin. ×1300 (*a*, *b*), 620 (*d*).

ture runs a very favorable and rapid course causing no death or dissemination of the process.

Foci of *Candida*-induced pneumonia were clearly discernible 6-9 h postinoculation, the peak of the inflammatory, mostly leukocytic, reaction was observed by 24 h postinfection, and complete regression and sanitation of the focus by days 5-7. Microscopy showed numerous leukocytes with an admixture of macrophages filling the lumens of alveoli and bronchi at the peak of pneumonia, these cells actively phagocytosing the fungal cells (Fig. 1, *a*).

Examination of semithin slices showed individual free-lying fungal blastospores and, still more seldom, short growth tubes amidst dense cell infiltrate (Fig. 1, *b*); no viable, let alone dividing fungi were detected by histoautoradiography. The fungi phagocytosed by macrophages and leukocytes are as a rule digested, but phagocyte cell walls are characterized by a very high resistance to proteolytic enzymes for a long time (Fig. 1, *c*).

In the course of sanitation of a focus its cellular composition is gradually replaced: on day 1 postinfection the macrophage to leukocyte ratio is 1/4, until by day 4 macrophages represent up to 80-85% of the inflammatory exudate cells. Multinuclear cells are formed in the focus of inflammation after 36 to 48 h, and these cells actively participate in phagocytosis. We have been unable to find published reports about so early an appearance of giant cells and their contribution to an acute inflammatory reaction.

Special attention should be paid to the role mast cells play in the sanitation of pneumonia foci. During the first hours postinoculation these cells may be seen in the perivascular and peribronchial connective tissue, and after 12-20 h they migrate to alveolar lumens and participate in the acute inflammatory reaction by active degranulation (Fig. 1, *d*). As the exudate changes, mainly from leukocytic to macrophagal, mast cells disappear.

No sanitation of *Candida* pneumonia foci by a granulomatous reaction occurred in animals with an intact immune system.

Combined drug treatment was carried out by two-week intramuscular injections of cyclophosphamide in a dose of 30 mg/kg b. w. every other day and of the broad-spectrum antibiotic ampiox in a dose of 40 mg/kg 3 days before inoculation. Both drugs were then injected in parallel, the former every other day, the latter daily during the entire course of observation (30 days).

Mycotic involvement of the lungs developing after intranasal infection of guinea pigs following drug treatment was characterized by early development, massive destruction of lung parenchyma and bronchial walls, a weakly expressed exudative reaction, and the formation of large foci of pneumonia containing numerous elements of pseudomycelium.

The degree of immunodepression prior to the experiment was assessed by blood analysis: the granulocyte count at the time of inoculation was less than 1000/mm. The thymus was the most vulnerable organ for cyclophosphamide: it drastically shrank, its separation into medullary and cortical substance was lost, the number and size of Hassall's bodies decreased to the point where they completely disappeared and the lymphoid tissue was reduced (Fig. 2, *a*). The lymph nodes (particularly regional) and spleen exhibited lymphoid tissue reduction with degradation and a sharp decrease in the count of lymphocytes and the size of follicles, as well as sinusoidal histiocytosis.

Whereas only adhesion of fungal blastospores to alveolar walls and bronchocyte surface without apparent damage to them, as well as a poorly expressed exudative reaction may be observed during the first 3 h after inoculation, after 6 hours the fungi start to multiply vigorously, colonizing and invading the pseudomycelium in the bronchial walls with complete desquamation and destruction of the bronchothelium (Fig. 2, *b*).

An inflammatory process develops in the lung parenchyma both as a result of perforating growth through the bronchial walls with impairment of the peribronchial lung tissue and directly in the respiratory portions from initially aspirated fungal cells (Fig. 2, *c*). The peak of the exudative reaction is observed 48 h postinoculation.

Macroscopically, at this stage, numerous gray foci 3 to 5 mm in diameter are seen in the lungs; microscopically these foci correspond to pneumonia foci in which a sluggish, mainly leukocytic, inflammatory reaction is attended by colonization of fungi with perforating growth through not only alveolar septa and the bronchial tree, but the walls of large vessels and capillaries as well (Fig. 2, *d*). The microcirculatory bed is dilated, and the capillary lumens are lined with fibrin clots (Fig. 2, *d*). Such a peculiar reaction on the part of the lungs evidently represents a specific mechanism of host defense, which is aimed at limiting the focus of inflammation and prevents dissemination of the fungi. Although fungi were frequently found in vascular lumens, we never observed mycotic foci in other organs. The cellular composition of the exudate is mainly leukocytic, with macrophages constituting less than 10% and mast and multinuclear cells absent altogether.

Electron microscopy showed that phagocytosis in this process is in general incomplete, with fungi

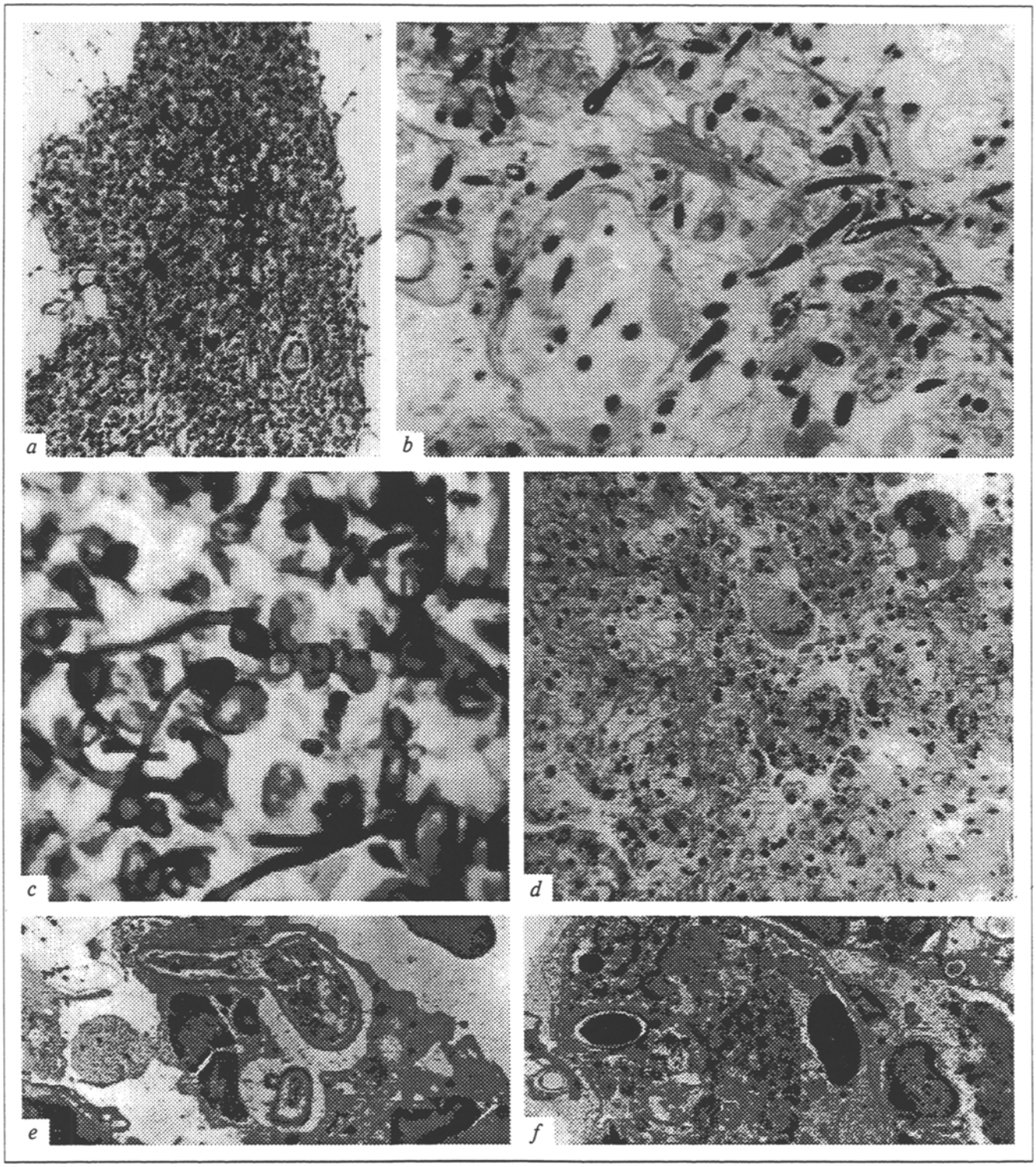

Fig. 2. Morphological picture of a focus of *Candida*—induced pneumonia during combined drug treatment. *a*) thymus after two—week cyclophosphamide injections. Manifest reduction of lymphoid tissue, disappearance of Hassall's bodies. Semithin slice. Hematoxylin—eosin, ×150; *b*) pseudomycelium invasion in bronchial wall, perforating growth in the vessels. Semithin slice. Methylene blue—basic fuchsin, ×620; *c*) abundant colonization of fungi in alveolar lumen. Day 1 postinfection. PAS reaction with hematoxylin counterstaining, ×620; *d*) day 8 postinfection. Giant—cell pneumonia. Semithin slice. Methylene blue—basic fuchsin, ×240. *e*) fungal cell growth in phagocyte lumen. Electronogram, ×7200; *f*) fungal cells and fibrin clot in capillary lumen. Electronogram, ×29,000.

growing in abundance in the phagocyte cytoplasm (Fig. 2, *e*).

As a rule, animals died after 2 to 5 days, the mortality being 35-40%. Viable fungal cells persisted

in pneumonic foci up to days 7-10, this being confirmed by their active incorporation of the RNA precursor uridine labeled with tritium.

Up to day 5 the fungi are not only viable, but actively propagating, which fact is confirmed by their utilization of a DNA precursor. The degree of isotope incorporation gradually decreases (a detailed histoautoradiographic picture of foci of candidal pneumonia was described previously [8]) and is terminated by days 7-10.

This period marks the beginning of sanitation and organization of a focus of pneumonia, which may take two directions: in 25% of cases it is the formation of granulomas with their subsequent complete cicatrization. A more frequent variant is the development of giant-cell pneumonia leading to pseudocarnification (Fig. 2, *f*).

After the fungi are completely eliminated from the lung parenchyma, numerous spherical lipid inclusions are observed in the macrophage cytoplasm, which fuse to destroy phagocytes and lie freely in the alveolar lumens, their size reaching that of giant multinuclear cells. These accumulations represent the product of massive fungal degradation. Electron microscopy showed similar inclusions in fungal cells.

The pathomorphogenesis of experimental candidal pneumonia under conditions of immunodepression and antibacterial therapy fits neatly into the modern concepts of the time course of this process under clinical conditions and includes fulminant colonization and invasion of the agent with its penetration into the vascular bed and possible subsequent dissemination of the process. If the immune system is intact, the infectious process confines itself to interactions between the agent and defense host cells, whose reactions effectively block the invasion and propagation of the agent.

Histoautoradiographic examination of foci of pneumonia makes it possible to assess both the morphology and function of the agent in the foci and adjacent tissue, which may help predict the further course of the process; a progressive increase of the intensity of DNA and RNA precursor incorporation is a poor prognostic sign.

Lipid drops detected in large quantities in sanitized foci of candidal pneumonia may serve, in the absence of the agent, as a distinctive marker of a prior *Candida* infection.

A developing secondary immunodeficiency not only boosts colonization and increases the depth of invasion, but also changes the type and degree of tissue and defense cellular reactions both in the acute period and during sanitation of the focus.

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