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MYCOTIC INFECTIONS COMPLICATING HEROIN ADDICTS, AIDS AND OTHER IMMUNOCOMPROMISED HOST CONDITIONS

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Summary.– *Mycoses due to opportunistic yeasts (*Candida albicans, Cryptococcus neoformans*) or filamentous fungi (*Aspergillus fumigatus*) on immunocompromised host conditions in patients with primary diseases are well-known from many years in patients with primarily diseases with granulocytopenia cellular and humoral defects or patients treated by immunosuppressive therapy, but since 1980 new aspects of mycotic pathology appeared in heroin addicts and AIDS patients. We have observed a new pathologic septicemic syndrome of* C. albicans *infection characterized by fever followed immediately by cutaneous disseminated lesions, ocular metastasis and later after several weeks by osteoarticular involvement. The apparition of this new pathologic syndrome might be related to a depressive action on cellular immunity. Mycoses are present in high proportions and play an important part in clinical picture of AIDS.* C. albicans *producing oral thrush and esophagitis is the most frequent with a large incidence (50 and 90%) with benign evolution generally; on the contrary,* Cryptococcus neoformans *responsible of disseminated, lymphadenic, meningoencephalitic, pulmonary or other deep localizations is less frequent (6 to 10%), but often with a extremely severe evolution, to rapid fatal issue in absence of specific antifungal therapy. A high virulent pathogenic fungus* Histoplasma capsulatum, *from endemic areas, usually not considered as opportunistic fungus, should be added to the list of agents of opportunistic infections in AIDS, nevertheless with a reduced incidence. Other fungi were recently recognized as agents of mycotic infections in immunosuppressed patients.*

Riassunto (Le micosi opportuniste nei tossicodipendenti, nei soggetti affetti da AIDS e negli immunocompromessi). – *Le micosi dovute ai lieviti opportunisti (*Candida albicans, Cryptococcus neoformans*) o ai funghi filamentosi (*Aspergillus fumigatus*) nell'ospite immunocompromesso per malattia primaria sono diagnosticate da molti anni in pazienti con granulocitopenia, deficit umorale o cellulare o pazienti in terapia immunosoppressiva, ma dal 1980 si sono manifestati nuovi aspetti della patologia da miceti nei tossicodipendenti e nei soggetti affetti da AIDS. Noi abbiamo potuto osservare una nuova sindrome settice-mica nell'infezione da* Candida, *caratterizzata da febbre, seguita molto precocemente da lesioni cutanee disseminate, metastasi oculari e, dopo diverse settimane, da un interessamento osteoarticolare. È possibile che tale sindrome sia correlata ad una azione depressiva sull'immunità cellulare. Le micosi mostrano una elevata incidenza nell'AIDS del cui quadro clinico rappresentano un aspetto importante. La specie più frequente (50-90%) è* Candida albicans, *causa di mughetto orale ed esofagite, generalmente ad andamento benigno; al contrario,* Cryptococcus neoformans *responsabile di infezioni disseminate, linfoadeniti, infezioni meningoencefaliche, polmonari o altre localizzazioni profonde, è meno frequente (6-10%), ma spesso con una evoluzione molto grave, fino ad un esito letale in mancanza di una specifica terapia antimicotica. Un micete patogeno altamente virulento* Histoplasma capsulatum, *proveniente da zone endemiche, generalmente non considerato opportunista, dovrebbe essere aggiunto alla lista degli agenti opportunisti nell'AIDS, anche se con una incidenza molto bassa. Recentemente altri funghi sono stati riconosciuti come agenti di infezioni micotiche nei pazienti immunocompromessi.*

Introduction

Over the past few years, the profile of fungal infections has taken a new appearance reflected in the higher incidence of deep mycoses affecting the viscera, generalization and septicemia due to new pathological conditions particularly in immunocompromised host conditions.

The principal fungi responsible for deep, systemic or visceral mycoses are:

a) opportunistic cosmopolitan yeasts (*Candida, Cryptococcus, Torulopsis, Trichosporon*, etc.) producing candidosis, cryptococcosis and other yeast infections and filamentous fungi (*Aspergillus, Mucor, Rhizopus*, etc.) producing aspergillosis, mucormycoses, etc. All these fungi, with the exception of *Candida albicans*, an endogenous yeastlike fungus living normally only in the digestive tract, are exogenous saprophytes in nature (soil, plants, air, water) and penetrate into the host when favorable conditions are realised;

b) highly pathogenic dimorphic fungi (*Histoplasma capsulatum, Coccidioides immitis, Blastomyces dermatitidis*, etc.), responsible for systemic mycoses as histoplasmosis, blastomycosis, coccidioidomycosis, etc. The spores of these fungi are in the soil of well defined geographical area, particularly in some tropical or subtropical regions and produce disease when they are introduced in the respiratory tract in normal patients but recently some of these systemic mycoses, as histoplasmosis developed severe dissemination even with fatal issue in immunocompromised host [1], particularly in AIDS [2], and we wonder whether it should not be considered as an opportunistic mycosis in such conditions;

c) fungi, agents of subcutaneous or osteoarticular mycoses inoculated by traumatism as sporotrichosis, mycetoma, chromoblastomycoses, pheohyphomycoses; the last two diseases are capable to give some visceral localisations as cerebral abscesses in some conditions.

Among the favorable factors for fungal infections to develop the intrinsic factors depending on the host have an increasing importance. Beside the physiological factors such as age or pregnancy due to immunologic and endocrinologic conditions, the pathological factors are most important. For a long time it has been known that normally saprophytic fungi may invade patients with severe, debilitating, primary diseases like diabetes (favorable to the growth of *Candida* and *Mucor*), malignant blood diseases, Hodgkin's disease and various neoplasma (favorable to the growth of *Candida, Aspergillus, Mucor, Cryptococcus neoformans*). However, before the advent of antibiotic and corticosteroid treatments, these mycoses were found only very rarely in patients in the final stage of serious microbial infections, severe diabetes or other endocrinopathies, or malignant diseases. At present these secondary opportunistic fungal infections, are extremely common in an immunocompromised host, particularly with a granulocytopenia below 1000 elements/ml, as a sequel to antibiotic treatment in the case of *Candida* species, or to corticosteroid and immunosuppressive treatment in the case of other opportunistic fungi (*Aspergillus, C. neoformans, Mucor*, etc.), but also in the case of *Candida* or other yeast-like species to a lesser degree. Sometimes the fungal infection is diagnosed before the primary disorder and the mycosis becomes an indicative «signal» disease as cryptococcosis. The increasing problem of fungal infections is pointed out in recent studies [3]. Invasive fungal infections have been reported in the last years in 25% of patients who are chronically and intensively immunosuppressed by reason of underlying disease and drug therapy. The incidence of aspergillosis rises from 1.91 to 4.8 per million persons (+ 158%) and the incidence of *C. neoformans* infections rises from 1.3 per million to 2.3 per million (+ 78%) for the period 1970-1976 in USA, but the incidence rate is substantially higher in 1985 adding the opportunistic infections such cryptococcosis, candidosis and histoplasmosis occurring in AIDS.

The alterations of the immunological systems know new developments in last years. The pathogenesis of fungal infections occuring in immunocompromised host has been recently reviewed and discussed in the general context of microbial infections [4]. The non specific immune mechanisms including cellular (e.g., phagocytic leukocytes) and humoral (e.g., immunoglobulin production, complement systems) and the specific cell mediated and humoral immunity play important but unequal roles in resistance of fungal infections in the immunocompromised host.

The disorders of phagocyte functions due to a reduced number particularly of polymorphonuclears (granulopenia), neutrophil heterogeneity, cellular defects of phagocytes chemotaxis concerning adherence, deformability, direct or indirect migration, impaired chemotaxis related to abnormal microtubules, disorders of phagocyte killing including disorders of neutrophil granule formation and degranulation, and disorders of intraleukocytic metabolism are particularly involved in fungal infections such as candidosis, aspergillosis, but also in other opportunistic mycoses.

The chronic granulomatous disease related to an primary defect of polymorphonuclear leucocyte is often associated with invasive aspergillosis and rises the problem of an efficient chemotherapy.

The acquired immune deficiency syndrome (AIDS) constitutes a new condition for development of mycoses. Severe fungal opportunistic infections have been recently reported in USA, Europa, Africa and other countries [5, 6]. Essentially, candidosis particularly thrush and esophagitis [7], cryptococcosis [8] and histoplasmosis [1, 2, 9, 10] are concerned because the mediated immunity; other opportunistic infections in immunocompromised host as diffuse aspergillosis are exceptional in AIDS, their development being related to the absence or to the worse function of polymorphonuclear leucocytes.

Although histoplasmosis may occur in immunocompetent patients, we wonder, as do other authors [1, 2, 9, 10] whether it should not be considered as one of the major infection in patients with AIDS who had stayed in endemic area.

Among the extrinsic, iatrogenic factors influencing the host we distinguish drug factors favoring fungal infections and the interrupted integument due to medical and surgical interventions, which permit the penetration of opportunistic fungi even of true pathogenic fungi from the environment.

The role of antibiotics in the pathogenesis of *Candida* infections is based not only on yeast selection in the alimentary canal and mucous membranes (unbalance between bacterial flora and yeast flora in favor of the latter) but also on the repressive action of antibiotics on the immunological defense systems, resulting in decreased antibody production, repression of phagocytosis, and metabolic alteration of the host cells. The adhesion of *C. albicans* cells to surface structures of epithelial mucosal cells on mannan receptor sites is implicated recently according to arious new data to explain the favoring factor represented by antibacterial antibiotics. So, under the action of antibacterial antibiotics, the adherence of *C. albicans* is facilitated and the transition of *C. albicans* from yeast to the filamentous aggressive form is obtained *in vivo* and *in vitro* in few hours; this can explain the *in vivo* remarquable activity of azoles as ketoconazole, capable to stop the mycelial development at very small amounts [11].

Heroin addicts are a population at risk for certain infectious diseases such as bacterial and fungal endocarditis, hepatitis B, but since 1980 we have observed [12, 13] a new pathologic septicemic syndrome of *C. albicans* infection, which seems to be related to a depressive action on cellular immunity, characterized by fever, followed immediately by cutaneous disseminated lesions of folliculitis, pustulosis, subcutaneous nodules, ocular metastasis (retinitis, chorioretinitis, uveitis) and later after several weeks by osteoarticular involvement (spondylodiscitis, costochondritis); the new heroin involved, called Iranian brown heroin, does not contain *C. albicans*.

Mycoses in heroin addiction

Since 1940 [14] when Joachim and Polayes reported the first case of *Candida* endocarditis as complication of heroin addiction, numerous cases of fungal endocarditis and endophthalmitis were reported after intravenous drug abuse [14-17]; in Europe an Italian case of *Candida* myocarditis was observed [18]. On a total of 319 cases of fungal endocarditis reviewed recently 25% are observed among heroin abusers. The majority are due to *Candida* species and less to *Aspergillus* and exceptionally the other fungi. Among 55 cases of *Candida* endocarditis in drug addicts, *C. albicans* was isolated in 5 cases, *Candida* spp. other than *C. albicans* in 46 cases and *Candida* sp. in 4 cases. In contrast, in endophthalmitis, *C. albicans* is observed exclusively among heroin users, as well as in the new septicemic cutaneous, ocular and osteoarticular syndrome of candidosis that we observed since 1980 [12, 13].

Effectively since June 1980, we observed in Paris region among the heroin addicts a febrile septicemic syndrome with elevated temperature, chills severe headache, profuse sweating following 2 to 24 hours after drug injection; this episode of 1-3 days was followed by metastatic cutaneous lesions (disseminated folliculitis or pustulosis in hairy zones, deep seated scalp nodules) and by ocular localizations (mainly chrorioretinitis) appearing generally between 3-5 days. Lately, between 15 days and 5 months, osteoarticular lesions (vertebrae, costal cartilages, knees and sacroiliac) particularly spondylodiscitis. The disseminated cutaneous lesions associated to ocular and osteoarticular lesions have not previously been described in classical systemic candidosis; we also observed hair invasion by candidal hyphae, the aggressive form of *C. albicans*, the only species found in this syndrome. *C. albicans* was not found in the heroin samples analysed; the outbreak of this form of candidosis that we described in details in 38 cases [13] but which is evaluated actually at more than 200 cases coincided with the introduction of a new brown heroin on the drug market in the Paris area. *C. albicans* was not isolated from the drug in our studies [13] as well as in Italian samples of heroin [19] but was very likely introduced by a contaminated serringe from a common spoon in which the crude heroin is dissolved; effectively all patients have oral and digestive candidosis. The apparition of this new pathologic syndrome might be related to a depressive action on cellular immunity; alterations of T and null lymphocytes frequencies in the peripheral blood of human opiate addicts were observed with *in vivo* evidence for opiate receptor sites on T lymphocytes [13]. An immunosuppressor contaminant of the brown heroin may also be the origin of this new pathology characterized by the sudden transformation of the yeast of *C. albicans* into the mycelial aggressive form involving the hair of the pilous follicles, the retina and the vertebral disc [13]. This candidosis syndrome was observed also in other regions of France, in Italy [17, 19] in Spain [20, 21], even in Australia [22], but not yet in USA.

Mycoses in AIDS

Among the opportunistic fungi implicated in AIDS, the yeast-like fungus *C. albicans* with oral thrush and esophagitis as clinical manifestations is the most frequent with a large incidence which varies between 50 and 90% according to the risk groups with benign evolution generally; on the contrary, *C. neoformans* responsible of disseminated, lymphadenic, meningoencephalitic, pulmonary or other deep localizations is less frequent (6 to 10%), but often led to extremely severe evolution, to rapid fatal issue in absence of specific antifungal treatment.

Other opportunistic fungi as filamentous molds such as *Aspergillus fumigatus* have a weak incidence

(0,16%) due to the fact that their development is related chiefly to the neutrophilic functions.

High virulent pathogenic fungi *Histoplasma capsulatum*, from endemic areas, usually not considered as opportunistic fungi, should be added to the list of agents of opportunistic infections in AIDS, nevertheless with a reduced incidence.

Among the Actinomycetes, *Nocardia asteroides* represents the most frequent agent although the pulmonary infection that it provokes has a low incidence.

Only two extended surveys of the available literature until 1984 were conducted on mycoses in cases of AIDS worldwide [6, 7] based on 3170 cases of AIDS reported to the CDC since 1981 on June 1984 [23], figure increased with 319 patients in 11 European countries and 57 African patients [24]. The total number of AIDS cases increased progressively to more than 15000 cases in USA at the end of 1985 and 2006 cases reported in Europe [25] by 21 European countries: the total number of AIDS cases by country which was for instance 573 for France, 377 for West Germany, 287 for Great Britain, 140 for Italy, 100 for Switzerland, 68 for Denmark, is contrasting with the number of AIDS by millions of inhabitants: 10.4 for France, 6.2 for West Germany, 5.1 for Great Britain, 2.4 for Italy, 15.4 for Switzerland, 13.3 for Denmark. A large number of authors reported anecdotical cases of mycoses based on restricted series; exceptionally detailed statistics focalized only on a mycotic disease as cryptococcosis, were surveyed only in one country as was done in France by the French Society of Medical Mycology [8] which showed that out 49 cases of cryptococcosis in 1985, 23 (46%) were associated with AIDS.

Candidosis

Candidosis appears as the principal opportunistic infection in AIDS and pre-AIDS individuals as well in American, Haitian, Danish, French, or African patients. Candidal infection in these patients usually is limited to the oropharyngeal, esophageal and gastrointestinal mucosa; exceptionally can also be disseminated (3 cases in American series) or pulmonary (4 cases including one pulmonary abscess). It is now well established that *C. albicans* may depress the T-cell immune system [26]. The frequency with which unexplained oral candidosis led to unequivocal acquired immunodeficiency syndrome (AIDS) was studied in patients at risk [27]. The authors conclude that in patients at high risk for AIDS, the presence of unexplained oral candidosis predicts the development of serious opportunistic infections more than 50 per cent of the time. Whether the remainder will have AIDS is not yet known. It is possible that the fungal antigen (mannan) of *C. albicans* can enhance the immune deficiency as was shown in chronic mucocutaneous candidosis [28, 31]. Only a strong antifungal therapy based on the use of amphotericin B in perfusion, or ketoconazole by oral route might suppress the antigen production and improve the cellular immunity. It is surprising that the systemic, renal and other deep manifestations of *C. albicans* are rare in AIDS; humoral immunity related to B lymphocytes or innate mechanisms may play a decisive role in protecting patients against these manifestations of candidosis.

The classical treatment of thrush and other *C. albicans* localizations of the digestive mucosae is based on oral polyene antibiotics (nystatin, amphotericin B) [26] and oral ketoconazole [31] but for AIDS, fluconazole (FCZ), a new triazole antifungal agent [29] seems remarquably effective. The original features of this triazole are: water solubility, long half life: 24 h, low serum protein binding and diffusion in CSF and urine. FCZ was given orally –50 mg once a day- in oral candidosis in twenty LAV/HTVIII antibody positive patients with excellent clinical and mycological results. To avoid relapses at present to recommended therapy is 50 mg daily during 5 days, followed by 50 mg every other day.

Cryptococcosis

This mycosis is the second most common fungal infection reported in AIDS, but it is the most severe opportunistic complication particularly when cryptococcal meningoencephalitis and septicemia occurred. Really, *C. neoformans* a saprophytic yeast largely prevalent in nature since its discovery in 1895 by Sanfelice in Italy, became highly pathogen in the immunocompromised host by some primary diseases, immunosuppressive treatments, organ transplantations. This yeast is surrounded particularly *in vivo* by a large polysaccharidic capsule responsible of the altered cellular immunity, paralysis or immunitary tolerance. Since the study by Drouhet *et al.* [30] in 1950, showing that the capsular polysaccharide constitued by a galactoxylomannan is a virulence factor inhibiting migration of leucocytes [31], numerous studies on the capsular antigens followed and led to the discovery of 4 serotypes A, B, C, D; they correspond to 2 sexual forms of this basidiomycetous fungus, with biochemical, epidemiologic and pathologic differences. The quantity of capsular material produced in the host may be so great that it can be found as free, circulant antigen in the body fluids, easily detected by a latex particules agglutination test. The surprising low incidence of this opportunistic mycosis (named «European blastomycosis» at the beginning of its history) implies that natural cell mediated resistance may be a major contributing factor to host defence but in the last decade this «sleeping mycosis» as called by Ajello in 1970, became so frequent in USA that it was named the «awakening giant» by Kaufman [32] in 1979: from 300 cases reported between 1965 and 1977, the total number observed only in USA between 1965 and

1977 rises to 1254 cases. Since 1981, the relation between AIDS and cryptococcosis increased progressively the number of cryptococcal infections: on a cumulative number of 13,834 cases of AIDS reported in October 1985 by CDC, 904 cases were invaded by cryptococcosis.

The detailed reports were published in small groups of AIDS patients showing a prevalence between 2-6%; only recent reports treat larger series [33-35]; this mycosis was reported particularly in USA among Haitian immigrants [36] and associated to AIDS not only in adult homosexual groups of risk but also in heterosexual groups, in drug abusers, in women and children. Central Africa is an endemic zone for the etiological agent of AIDS [39]. For about 2 years there was a sharp increase in cryptococcosis in the major hospitals of Kinshasa [37-39]. The tropical foci of AIDS from Haiti and Central Africa were confirmed and immigrants form Haiti and black African with AIDS and cryptococcosis were observed in Europe particularly in France [8, 40] and Belgium [41].

In France, the number of cases of cryptococcosis diagnosed, confirmed by laboratory data, by the Mycology Unit at the Pasteur Institute was 5 in 1980, 7 in 1981 and rises suddenly to 17 in 1982, 19 in 1983, 19 in 1984 and 24 in 1985. Half of them since 1982 are associated with AIDS [8].

Among the large series of cryptococcosis in USA, Kovacs *et al.* [33] reported from 6 medical American centers the clinical course and response in therapy of 27 patients with cryptococcosis and AIDS observed between January 1979 and October 1984. Standard courses of amphotericin B alone or combined with flucytosine were ineffective. Cryptococcosis in patients with this syndrome is a debilitating disease that does not respond to conventional therapy; earlier diagnosis or longterm suppressive therapy may improve the prognosis.

Zuger *et al.* [35] observed 34 cases (9%) of cryptococcosis among 396 patients with AIDS. Twenty-two patients had brain or meningeal disease; the others had pulmonary disease (2 patients), pericarditis (1 patient), and antigenemia (1 patient). During treatment, 3 patients died of cryptococcosis and 3 died of other causes. Fifteen patients were folllowed for more than 6 weeks after amphotericin B treatment. The maintenance therapy with amphotericin B may be needed to prevent relapse in patients with AIDS.

In France a detailed epidemiological study on cryptococcosis was conducted by the French Society of Medical Mycology with the cooperation of the numbers of the French Society of infectious diseases and reported by B. Dupont [29]. Forty-nine cases were recorded, in all cases *C. neoformans* was grown except in one case with high level antigenemia. Sex ratio was male 39, female 10. Mean age was 39.8 years in 47 adults (range 17-72 years). Two children were 10 and 16 years old. Racial data showed that 68.7% of cases were Caucasian from Europe, 8% were coloured, 10,4% were Arabic from North Africa or came from Asia or South America. Race was not available in one case. Distribution: 28 cases in the Paris area, 21 cases in other main towns of the country. Birds may have played a role in 4 cases: pigeon breeding: 1 case, birds at home: 3 cases (parrot : 2, canary: 1).

Recorded predisposing factors are following:

AIDS	23
Hodgkin, lymphoma	4
Malignant haemopathia	3
Kidney transplant	3
Systemic lupus erythematous, diabetes mellitus, pemphigus, cancer, sarcoidosis, traumatism	1 each
None	9
Not available	1

Twelve patients from the first six in the above groups were receiving corticosteroids and/or chemotherapy. Main localizations were:

Central nervous system	90.9%
Lung and pleura	37.7%
Liver	12.7%
Skin and soft tissue	8.3%
Urine	4.2%

46.8% of patients had two or more localizations of the disease. Blood cultures were positive in 34% of patients. *In vitro* resistance to antifungal agents was limited to flucytosine in 13.5% of the cases. Cryptococcal antigen in spinal fluid was assayed in 26 patients and found present in 22 who were also positive for *C. neoformans* and negative in 4 patients: 1 cutaneous form, 2 brain abcesses and 1 patient with antigenemia and antigenuria without isolation of *C. neoformans*. 25 strains were serotyped at the Mycology Unit in the Pasteur Institute: 24 were A or AD, 1 was C in a man originated from Cambodia [40]. All the strains from AIDS patients of our series are of A serotype, in spite of the fact that some patients are from regions where serotype B-C was observed [47]. AIDS is a new predisposing factor since 1982 in France and account for almost half the cases. Most patients are male: in France most AIDS cases occur in homosexual people.

In 8 coloured patients with cryptococcosis in this series 7 have AIDS. Lung biopsies are good tools for diagnosis, antigen titers may be very high. Positive blood cultures are equally frequent in AIDS or non AIDS patients. Biological, clinical and therapeutical data on 16 patients observed in France were recently reported [34]. In Italy, Viviani and Tortorano [41, 42] reported for a period of 14 months (1984-1985) 6 cases of AIDS with cryptococcosis. A prevalence of 8.6% (8 cases on 92 AIDS) was established by the

Italian system of control of AIDS (Istituto Superiore di Sanità) on the basis of cases reported in Italy until October 20th 1985. These data are including only 25 of the 58 cases of AIDS reported at Assessorato alla Sanità della regione Lombardia; in this region the cryptococcosis has a high prevalence (17.2%). Itraconazole, a new oral azole, was tried in two cases with preliminary favorable results, but the patients are still under treatment and is too early to have a definite response. A case of inoculation of cryptococcosis without transmission of the acquired immunodeficiency syndrome was reported [41, 42].

Histoplasmosis

Histoplasmosis due to *Histoplasma capsulatum*, a true pathogenic fungi is not considered to be strictly an opportunistic infection, nevertheless more recently cases of histoplasmosis were reported among immunocompromised patients from either systemic malignancy or steroid therapy [1]; cell-mediated immunity is thought to be responsible for limiting proliferation of *H. capsulatum* in tissue. Recent attention has been focused on several cases of histoplasmosis occurring in AIDS patients reported in USA [43, 44] or in France [2, 9] from patients living or travelling in geographical endemic area of histoplasma. Four cases of histoplasmosis in AIDS were observed in France.

In one of them *H. capsulatum* was observed in a peripheral blood smear [9] of a patient originary from Haiti contaminated in French Guyana; this case ressembles to an other case reported in a patient with AIDS observed in USA [45]. In both cases *H. capsulatum* yeasts were seen in blood smears into the polymorphonuclears. A fourth case is a woman, 37 years old, originary from Zaire presenting in France a disseminated histoplasmosis; *H. capsulatum* was isolated from liver, lymphonodes, bone-marrow and bronchoalveolar lavage [38].

In a series of 14 young patients among heterosexual drug abusers [46] a Portorican man, 39 years old, using intravenous heroin presented disseminated histoplasmosis, oral thrush with association of Epstein - Barr virus. Kaur and Meyers [47] reported the case of a homosexual man treated with corticosteroids for severe idiopathic thrombocytopenic purpura who died from disseminated histoplasmosis. Autoimmune thrombocytopenia treated by corticosteroids and splenectomy might be a cause of risk in homosexual man.

In consequence, disseminated histoplasmosis might be suspected in patients with AIDS or the AIDS-related complex who have sepsis in the absence of bacterial, viral, and parasitic infections that frequently affect these patients [48, 49]. Serologic studies can be helpful, but in patients who are severely ill and seropositive, as our patients were, invasive diagnostic procedures such as lymphonode and bone marrow biopsy must be decided on quickly to permit the prompt initiation of antifungal therapy. As others, we conclude that disseminated histoplasmosis should be added to the list of opportunistic infections in AIDS.

Aspergillosis

This mycosis is more commonly associated with neutropenia than with lymphopenia and the role of T-cell mediated immunity, if any, seems to be secondary [50]. Schaffner [50] is wondering if disseminated aspergillosis is at least moderate by predictive of underlying cellular immune deficiency. Out of 3170 AIDS cases reported by CDC between May 1983 and June 1984, only five (0.16%) included invasive aspergillosis, reason for that CDC recently has deleted from the list of infections considered to be at least moderately predictive of AIDS [51]. Nevertheless a case of candidosis and aspergillosis due to *A. fumigatus* was reported recently [51] in a 32 years old man, drug abuser and AIDS patient. The patient died on the 15th hospital day.

Alternariosis

A case [52] is reported in a 7 years old girl with acute lymphoblastic leukaemia who developed a necrotic lesion of the left hand, from which *Alternaria* sp. was cultured. The lesion was associated with a varicella viral infection. The patient was later discovered to be suffering from transfusion-associated acquired immune deficiency syndrome (AIDS). The lesion was successfully treated with oral ketoconazole (10 mg/kg daily) plus local econazole.

Nocardiosis

A systemic infection due to aerobic Actinomycetes (*Nocardia asteroides*) with pulmonary localization has been observed in 6 of 3,170 AIDS.

Other mycoses and fungi from AIDS patients

Exceptionally pityrosporosis (*Pityrosporum* or *Malassezia ovale*), pityriasis versicolor (*Malassezia furfur*), *Torulopsis glabrata* infections were reported in AIDS.

From the monocytes of three patients with AIDS, atypical isolates of *Thermoascus crustaceus* (*Dactylomyces crustaceus*) were isolated [53]. The mycelium of these isolates contains a cyclosporin-like compound, which has been found also in four of four patients with AIDS. Four additional attemptes to culture fungus from AIDS patients have thus far been negative.

This fungus may simply be a contaminant of the monocytes cultures or another opportunistic infection in these patients, but the cyclosporine-like material may be also cofactor that cause the total impairment of the immune system seen in AIDS.

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ATTUALITÀ E PROSPETTIVE DELLA PROFILASSI ANTIFUNGINA NEI LEUCEMICI

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Riassunto. – *Parecchi importanti problemi della profilassi antifungina sono ancora irrisolti. Per quanto riguarda le micosi viscerali primitive, la ricerca è ancora alla fase iniziale. Nelle micosi opportunistiche buone possibilità esistono attualmente solo per le forme endogene da lieviti (chemioprofilassi orale). Nelle infezioni esogene (aerogene) solo un severo isolamento ambientale può risultare realmente efficace. Esso risulta tuttavia costoso e di difficile gestione. Alcuni farmaci antifungini (itraconazolo JANSSEN, UK-49858 PFIZER) offrono moderate speranze per una efficace chemioprofilassi in queste forme.*

Summary (Antifungal prophylaxis in leukemic patients). – *Important problems of antifungal prophylaxis do not appear to be resolved yet. The research concerning primary deep mycoses has just begun, while the opportunistic mycoses from endogenous yeasts are today successfully treated (oral chemoprophylaxis). Strict reverse isolation is the only treatment that seems to be really effective in the exogenous airborne infections. However, this prophylactic method is very expensive and difficult to manage. New antifungal drugs (itraconazole JANSSEN, UK-49858 PFIZER) seem hopeful for a complete antifungal chemoprophylaxis.*

La leucemia acuta può essere considerata un modello esemplare delle malattie esposte al rischio di superinfezione fungina. Da una parte, infatti, vengono in essa a coesistere fattori predisponenti molteplici, legati alla malattia di base o iatrogeni, che cooperano a deprimere in modo particolarmente severo le capacità di difesa dell'organismo; dall'altra, l'esprimersi della complicanza fungina è reso possibile e relativamente frequente dalla prolungata sopravvivenza che a molti pazienti è oggi concessa dai moderni protocolli antileucemici, specie per quanto riguarda la concreta possibilità di un efficace controllo delle complicanze emorragiche ed infettive batteriche, prima rapidamente fatali. Viene perciò sempre più avvertita la necessità di integrare i suddetti protocolli terapeutici con presidi profilattici antifungini.

A questo scopo possono essere presi in considerazione la chemioprofilassi e l'isolamento ambientale.

Per quanto riguarda la prima, mi sembra debbano essere sottolineati i punti seguenti:

1) A differenza delle complicanze batteriche, per contrastare le quali l'abbondanza di antibiotici a disposizione consente quasi sempre una adeguata modulazione del trattamento profilattico, che tenga conto delle diversità dei pazienti e, soprattutto, della popolazione microbica in causa (specie coinvolte, fenomeni di chemioresistenza, ecc.), il corredo di farmaci utilizzabili contro le specie fungine opportunistiche risulta circoscritto a poche molecole. Questa spiacevole realtà, alla quale non sembra possibile porre riparo in tempi brevi, deriva fondamentalmente, come è noto, dalle strette affinità strutturali che esistono fra cellule eucariotiche fungine e dei tessuti umani, affinità che ostacolano gravemente l'individuazione nel tallo fungino di bersagli metabolici abbastanza specifici, su cui possa esplicarsi in modo sufficientemente selettivo l'azione antibiotica.

2) Di questi pochi farmaci, nistatina ed amfotericina B (polienici) non possono essere utilizzati per una profilassi sistemica, rivolta cioè ad assicurare concentrazioni antibiotiche efficaci a livello dei tessuti. La nistatina è caratterizzata, infatti, da una tossicità molto elevata, che ne limita l'uso al trattamento orale delle discusse forme digestive, in quanto non assorbita dal tratto gastroenterico. Da parte sua l'amfotericina B, pur costituendo tuttora il farmaco di elezione nel trattamento sistemico delle micosi viscerali, presenta anch'essa un grado di tossicità non trascurabile, che ne giustifica l'uso solo per l'eradicazione, non altrimenti ottenibile, di infezioni fungine gravi.

3) Tuttavia, poiché ne manca l'assorbimento digestivo, ambedue questi farmaci sono ampiamente

utilizzati per via orale per il controllo limitativo della popolazione fungina lievitiforme frequente commensale del tratto digestivo; per la profilassi, cioè, delle micosi opportunistiche endogene (candidosi). Sull'efficacia, apprezzabile anche se non assoluta, di questa modalità profilattica esiste ormai un'ampia documentazione.

4) Un grado di tossicità nettamente inferiore a quello dei polieni caratterizza una seconda categoria di antifungini, a struttura imidazolica. Fra questi, miconazolo ed econazolo condividono con l'amfotericina B l'insufficiente assorbimento digestivo e l'efficacia terapeutica, sia pur complessivamente minore, quando siano somministrati per via endovenosa. In considerazione del grado più accettabile di tossicità il loro uso è stato proposto anche per la profilassi antifungina, con la presunzione teorica di estendere l'effetto protettivo alle infezioni esogene (aerogene), data la dimostrata possibilità di ottenere con essi concentrazioni antibiotiche persistenti ed efficaci nei tessuti.

In effetti il miconazolo rientra già nei protocolli profilattici di alcuni centri di ematologia, soprattutto nordamericani. La modalità obbligata di somministrazione endovenosa ne ostacola tuttavia l'uso sistematico in pazienti, come i leucemici, già a disagio per la molteplicità e gravosità dei trattamenti.

Il terzo farmaco imidazolico disponibile in commercio, il ketoconazolo, ha suscitato grandi speranze, anche in ambito profilattico, dopo che un'ampia casistica ne ha documentato l'efficacia terapeutica in molte micosi viscerali. A differenza dei precedenti imidazolici, infatti, il ketoconazolo presenta un buon assorbimento digestivo, che ne consente la somministrazione orale. Purtroppo esistono osservazioni, confermate anche dalla esperienza personale [1, 2], che indicano la presenza, proprio nei leucemici, di un suo quasi generalizzato difetto di assorbimento.

Di conseguenza le concentrazioni ematiche ottenibili risultano in essi abitualmente ridotte rispetto a quelle ottenibili in volontari sani ed in altre categorie di pazienti. Per quanto spesso compatibili con le concentrazioni minime inibenti (MIC) di molti lieviti, dette concentrazioni risultano perciò quasi sempre del tutto inadeguate rispetto alle MIC, tipicamente assai più elevate, delle specie saprofite ambientali.

In altre parole il ketoconazolo arricchisce e può forse migliorare, in certi casi, le possibilità profilattiche delle micosi endogene, peraltro già coperte in grado accettabile dalla profilassi con farmaci polienici, ma non risolve, se non in casi sporadici preventivamente individuabili, il problema delle micosi opportunistiche esogene.

5) L'ultimo farmaco disponibile, la 5-fluorocitosina, efficace *per os* e di tutti gli antifungini il meno tossico, non può essere utilizzato nella profilassi per la sua peculiare tendenza ad evocare fenomeni di chemioresistenza, oltre che per l'eccessivo volume del bolo alimentare richiesto (10-15 grosse compresse al giorno, come minimo).

Per quanto riguarda l'isolamento ambientale, l'esperienza personale mi induce a ritenere che esso possa risultare efficace nel prevenire le infezioni fungine esogene, anche senza ricorrere alle camere di isolamento a flusso laminare [3]. Nel corso di una prolungata collaborazione con l'Istituto di Ematologia dell'Università di Bologna, è stato infatti possibile constatare la completa assenza di micosi esogene nei pazienti ricoverati, nel corso di quasi cinque anni, nel reparto di isolamento a bassa carica microbica dell'Istituto; mentre, in un analogo periodo di tempo, ben cinque casi di aspergillosi viscerale ed uno di criptococcosi meningea sono stati osservati nei reparti aperti.

Per l'isolamento sono state assicurate le seguenti principali condizioni: ricovero in camere singole con gradiente positivo di pressione atmosferica fra camera di degenza ed ambienti comuni e fra questi ed il restante ambito dell'Istituto. Sterilizzazione preventiva delle camere di degenza con vapori di formaldeide. Filtrazione dell'aria. Sterilizzazione dei cibi e delle bevande. Trattamento preventivo con raggi ultravioletti dell'acqua di rubinetto lungo il decorso della tubatura di accesso al reparto. Trattamento periodico dell'aria ambientale dei vani comuni con areosols di iodofori. Cura particolare dell'igiene personale dei pazienti e degli addetti al reparto.

In conclusione, il problema della profilassi delle micosi opportunistiche nei leucemici, impostosi all'attenzione per l'incremento della loro frequenza, non può ancora essere considerato risolto. Particolari difficoltà permangono per quanto concerne la profilassi delle infezioni esogene (aerogene), la cui prevenzione esige la preesistenza di concentrazioni antibiotiche antifungine efficaci a livello dei tessuti ed in particolare di quello polmonare. Al contrario, abbastanza soddisfacente può essere considerato lo stato delle cose per quanto si riferisce alle micosi endogene, dal momento che già esiste una possibilità concreta di limitare lo sviluppo dei lieviti responsabili nella loro abituale sede di commensalismo, il tratto gastrointestinale.

Le speranze per l'ulteriore miglioramento di questa situazione e per il superamento definitivo delle difficoltà persistenti nella profilassi delle infezioni aerogene riposa sull'attuale, vivace attività di ricerca, volta a scoprire nuove molecole, che associno facilità di somministrazione, bassa tossicità e capacità di assicurare concentrazioni ematiche persistenti, stabili ed efficaci.

Qualche buona prospettiva in questo senso sembrano fornire due molecole di recente sintesi: l'itraconazolo, nuovo antifungino della Janssen, che realizzerebbe un rapporto particolarmente favorevole fra livelli di concentrazione ematica e MIC fungine, e l'UK-49858 PFIZER, alcool terziario triazolico, che sarebbe provvisto di alcune peculiari proprietà farmacocinetiche (tempi molto lunghi di emivita

plasmatica, scarsa affinità per le proteine del plasma, ecc).

Nelle infezioni fungine opportunistiche le possibilità di profilassi immunitaria restano allo stadio di ipotesi di lavoro. Nonostante sia difficile ipotizzare una risposta immunitaria efficace in soggetti, come i leucemici, per definizione immunodepressi, esistono tuttavia segnalazioni di effetti protettivi ottenuti nella candidosi sperimentale mediante vaccinazione specifica di topi pretrattati con ciclofosfamide [4, 5].

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LABORATORY DIAGNOSIS OF HERPES VIRUS INFECTIONS IN IMMUNOCOMPROMISED HOSTS

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Summary. – *The immune response to herpes simplex virus (HSV), varicella-zoster virus (VZV), cytomegalovirus (CMV), and to human fibroblast antigens (HF) was studied in six bone marrow recipients during immunosupressive therapy. The patients were followed for periods of 9 to 50 weeks, and serum samples were collected at approximately two weeks intervals. Antibodies of the immunoglobulin classes G (IgG) and M (IgM) were measured by solid-phase indirect enzyme immunoassay (EIA) using cell-lysate antigens. Sera were tested at a dilution of 1:100. IgM antibodies to one or more antigens could be detected in all but one patients during the time of observation, in two of them without a subsequent synthesis of specific IgG. But also significant increases of IgG levels in the absence of IgM antibody synthesis were observed. During chronic graft versus host reaction lasting approximately three months, one patient produced IgM antibodies to all the three viruses. Cellular components in the viral antigens are most likely responsible for this finding, since the patient produced large amounts of IgM class antibodies to human fibroblast antigens. Due to the impairment of immunological functions during immunosupressive therapy an interpretation of serological results obtained from such patients is difficult and requires a close cooperation betweeen clinicians and virologists. Other diagnostic possibilities for a laboratory diagnosis of herpes virus infections in immunocompromised hosts are discussed.*

Riassunto (Diagnosi di laboratorio delle infezioni da herpes virus nell'ospite immunocompromesso). – *È stata studiata la risposta immunitaria ad alcuni herpes virus (HSV, VZV, CMV) ed all'antigene fibroblastico umano (HF) di sei pazienti trapiantati con midollo osseo e sotto terapia immunosoppressiva. I pazienti sono stati seguiti per periodi di 9-50 settimane, raccogliendo i campioni di sangue ogni due settimane. Tramite tecniche immunoenzimatiche, sono stati rilevati anticorpi IgM contro uno o più antigeni in tutti i pazienti eccetto uno, in due di essi senza switch IgM-IgG. È stata anche osservata una aumentata sintesi IgG senza formazione di IgM. Un paziente ha prodotto anticorpi del tipo IgM contro tutt'e tre i virus durante una reazione di trapianto contro l'ospite, ad andamento cronico (circa tre mesi). La prevalenza di componenti cellulari nelle preparazioni antigeniche virali è la più probabile spiegazione di questi dati visto che i pazienti producevano grosse quantità di IgM contro gli antigeni fibroblastici. Pertanto, l'immunosoppressione presente in questi pazienti rende difficile l'interpretazione dei dati serologici ed è richiesta la stretta collaborazione fra clinico e microbiologo.*

Introduction

The laboratory diagnosis of viral infections bases either on the detection of the virus from any kind of clinical specimen, or on the measurement of the patient's immune response to the virus. Virus isolation, electron microscopy, detection of structural and nonstructural viral proteins by immunological and enzymatic methods and nucleic acid hybridization are the techniques available for the detection of viruses. Viral antibodies are detected by neutralization, complement fixation, indirect immunofluorescence and, more recently, different kind of immunoassays, which have found wide application in the serological diagnosis of viral infections.

Herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2), varicella-zoster virus (VZV), cytomegalovirus (CMV) and Epstein-Barr virus (EBV) form the group of human pathogen herpes viruses. With the exception of HSV-2, primary infection with herpes viruses usually occurs during childhood or early adolescence. In healthy individuals primary infection is usually asymptomatic or causes a mild disease that seldom requires a laboratory diagnosis. After primary infection herpes viruses are not completely

eliminated from the organism but enter a state of latency from which they can be reactivated and cause recurrent infection at any stage of future life.

Particularly patients under immunosuppressive therapy are at increased risk of getting recurrent, or if they have escaped earlier, primary infections with herpes viruses [1-3]. Both primary and recurrent infections can lead to serious, and often life threatening disease in the immunocompromised host. Since these patients may benefit from antiviral therapy [4], a laboratory confirmed diagnosis of the infection is essential. Virus isolation still is the most widely used technique for demonstration of viruses. The method is rather sensitive, however, for certain viruses, e.g. CMV and VZV it sometimes takes one to several weeks to obtain a conclusive result. HSV on the other hand, usually grows readily in cell cultures, and occasionally a result can be obtained within 24 hours.

Rapid methods that yield a result within hours are available for the diagnosis of vesicular rashes caused by HSV and VZV [5-7]. Viral antigens can be demonstrated directly from clinical specimens by immunofluorescence or by immunoassays. Immunofluorescence is by far the more rapid method but it requires reagents of highest quality and a large experience of the virologist to interprete the microscopic findings. Antigen detection by immunoassays offer the advantages that the tests are more objective than immunofluorescence and that the test result can easily be confirmed by blocking assays.

Recently, laboratory methods for the detection of CMV induced early antigens in infected cell cultures have been developed [8; 9]. Results by this technique can be obtained within 24 to 48 hours after arrival of the specimen in the laboratory and the sensitivity of the method is comparable to that of standard virus isolation. Also immunoassays for a detection of CMV antigens from clinical material without prior cell culture passage have been described. These kind of assays, however, are not yet readily available [10].

DNA hybridization is a novel and promising, culture independent tool for the identification of viruses from clinical specimens. The technique has been described for HSV, VZV, CMV and EBV, and with appropriate reagents, the method is of high specificity [11-16]. At the moment DNA hybridization is mainly performed with radiolabeled DNA probes. Biological hazards and short shelf life of radioisotopes may represent a disadvantage for using the technique in routine diagnostic laboratories, but further developments will show whether DNA hybridization will gain access as a routine method in diagnostic virology.

In immunocompetent hosts, serology, especially during primary infections, offers a useful possibility of getting a laboratory diagnosis of herpes virus infections. Most individuals develop specific IgM class antibodies early during the course of the infection, followed by the rise of IgG class antibodies. During reactivation of latent virus or during reinfection only part of the patients will develop IgM, and the rise in IgG may not be significant or may be delayed [17-19].

Due to the impairment of the patient's immune system under immunosuppressive therapy results from serological tests have to be evaluated and interpreted with great care and in close observation of the clinical picture.

Table 1 gives an overview over diagnostic possibilities available for the diagnosis of herpes virus infections of immunocompromised patients.

Materials and methods

Viruses and cells. – Viruses used in this study were CMV AD 169, HSV-1 strain G, and the OKA strain of VZV. All viruses have been originally obtained from the American type culture collection (Rockville, MD). CMV and VZV were propagated in human foreskin fibroblasts (HF), and HSV was grown in African green monkey kidney cells (VERO).

Preparation of antigens. – An envelope antigen from HSV infected VERO cells was prepared as described [20]. CMV and VZV infected cells were washed in phosphate buffered saline pH 7.4 (PBS), resuspended in PBS, submitted to three cycles of freezing and thawing, homogenized by sonication and finally the suspension was clarified by low speed centrifugation. The resulting supernatant was used as antigen. A similar antigen was prepared from uninfected HF cultures.

Patients. – Six patients were followed after bone marrow transplantation for a period of nine to 50 weeks. Transplantation was performed because of acute myeloic leucemia in five patients, and the remaining patient was transplanted for Burkitt's lymphoma. Three patients were females, and the mean age of all patients at transplantation was 28.5 years. Serum specimens were collected from these patients at approximately two weeks intervals and stored frozen at -20° C until tested.

Enzyme immunoassays. – Microtiter plates were coated with antigens at a concentration of 0.5 µg/well. Before use, plates were washed twice with PBS containing 0.01% Tween 20. Sera were diluted 1:100 in PBS supplemented with 5% normal porcine serum and 0.5% Tween 20 and incubated in duplicate wells for two hours at 37° C. After washing, peroxidase labeled anti-IgG or anti-IgM conjugates at appropriate dilutions were added and the plates were further incubated at 37° C for one hour. After washing, enzyme substrate and a colour indicator were added and the reaction was developed at room temperature in the dark for 30 min and then stopped by adding 1 N HCl. Optical densities were measured

Table 1. – *Selection of laboratory tests for the diagnosis of herpes virus infections in immunocompromised hosts. A combination of several methods is often advisable*

Virus	Symptoms	Specimen	Laboratory test	
			First choice	Second choice
HSV & VZV	vesicles	vesicle fluid vesicle crust basal cells	antigen detection	virus isolation
	oesophagitis	aspirate	virus isolation	antigen detection
	pneumonia	BAL (*a*)	virus isolation	antigen detection
	genital herpes	swab	virus isolation	antigen detection
	disseminated	throat swab	virus isolation	antigen detection
	infection	urine biopsy serum, CSF		IgG/IgM serology
	encephalitis	serum, CSF	IgG/IgM serology	
CMV	pneumonia	BAL (*a*) saliva buffy coat urine	early antigens	virus isolation
		serum		IgG/IgM serology
	fever, lymphadenitis, hepatitis	buffy coat saliva urine	early antigens	virus isolation
		serum		IgG/IgM serology
	encephalitis	serum, CSF	IgG/IgM serology	
EBV	any	serum, CSF	IgG/IgM serology	

(*a*) BAL = bronchoalveolar lavage

with a Titertek Multiskan® spectrophotometer (Eflab OY, Helsinki) at a wavelength of 492 nm. Known positive sera were included on each plate as a test control. A negative serum included in each test served as a reference serum. Test results were expressed as ratios calculated from the OD value obtained from the patient's serum and from the negative reference serum. A ratio of 2.5 or higher was regarded as a positive result. All sera were tested for IgG and IgM class antibodies to all four antigens on the same day.

Results

Between five and 20 sera were available for testing from each patient. Results obtained from these sera are presented in Table 2. All but one patient developed significant levels of IgM antibodies to one or more antigens. Patient No. 2 (Table 2) developed only borderline values of IgM class antibodies to HSV but to none of the other antigens during a 50 weeks period of observation, though CMV was isolated on several occasions from the patient's urine or saliva and from a bronchoalveolar lavage specimen at the end of the observation period. Two patients (No. 5 and 6, Table 2) showed a strong IgM response against HSV in the absence of detectable levels of IgG antibodies to this virus. Patient No. 5 had a vesicular rash during that time but neither HSV nor VZV could be detected from the vesicles. Patient No. 6, however, harboured HSV from her throat on several occasions during this study. Increase of IgG levels without simultaneous production of IgM was observed too (patient No. 3, HSV). CMV was isolated from patient No. 5 in the absence of IgM production and without IgG antibody increase.

Patient No. 4 developed IgM antibodies to HSV in the presence of constant high levels of IgG antibodies during the time of observation. The patient had an episode of recurrent genital HSV during that period, and HSV-2 was isolated from her urine and from vesicles on repeated occasions. VZV IgM was found in two, and CMV IgM in one of her sera without clinical or virological evidence of infection with these two viruses.

Over a period of three months patient No. 1 produced high levels of IgM antibodies to HSV, VZV, CMV, but also to HF antigens (Fig. 1). On six occasions during this phase CMV was isolated from urine and/or throat. Synthesis of CMV IgM antibodies still continued for several weeks after the production of IgM to HSV, VZV and HF had ceased. An increase of CMV IgG was noticed.

Table 2. – *Immune response of six bone marrow recipients to virus and cellular antigens after transplantation*

Patient age and sex	Weeks of observation	Number of specimens	Ig-class	Antigen											
				HSV			VZV			CMV			HF(c)		
				mean(b)	CV%(c)	range	mean	CV%	range	mean	CV%	range	mean	CV%	range
1. 36 y male	48	20	IgG	7.5	40.4	3.2-11.8	7.0	45.6	2.5-10.5	6.0	39.6	2.3-10.2	1.6	30.5	1.0-2.9
			IgM	4.3	43.4	1.9- 7.1	4.6	55.2	1.6- 9.3	4.5	60.2	1.9- 9.0	3.7	85.4	0.7-9.5
2. 14 y male	50	19	IgG	29.4	17.8	12.1-32.3	17.7	13.7	13.9-21.0	6.2	33.0	2.7- 9.1	1.2	65.2	0.1-3.0
			IgM	0.5	143.0	0 - 2.5	NPV(d)	—	—	NPV	—	—	NPV	—	—
3. 22 y female	9	6	IgG	14.6	37.1	4.2-19.5	20.5	4.7	19.4-22.1	16.2	15.8	13.7-21.0	NPV	—	—
			IgM	NPV	—	—	NPV	—	—	NPV	—	—	NPV	—	—
4. 40 y female	18	5	IgG	30.6	4.5	28.8-32.2	17.7	24.2	10.2-20.6	15.5	32.3	7.2-19.9	NPV	—	—
			IgM	4.0	117.1	0.2-10.7	2.2	48.2	1.0- 3.6	1.3	74.6	0.6- 3.0	NPV	—	—
5. 31 y male	10	7	IgG	NPV	—	—	16.3	11.8	12.5-18.6	4.8	19.9	3.5- 6.1	NPV	—	—
			IgM	11.5	77.7	0 -21.0	NPV	—	—	NPV	—	—	NPV	—	—
6. 28 y female	14	5	IgG	NPV	—	—	20.2	9.9	17.4-22.3	7.1	19.8	4.7- 8.1	NPV	—	—
			IgM	22.6	5.5	20.8-24.0	NPV	—	—	NPV	—	—	NPV	—	—

(*a*) HF = human fibroblast antigen
(*b*) mean = ratios were calculated from the OD value of the test serum and the OD value of a negative serum. Mean represents mean value obtained from all ratios of the sera tested. A ratio of 2.5 or higher is regarded as positive
(*c*) CV% = coefficient of variation
(*d*) NPV = no positive value during the period of observation

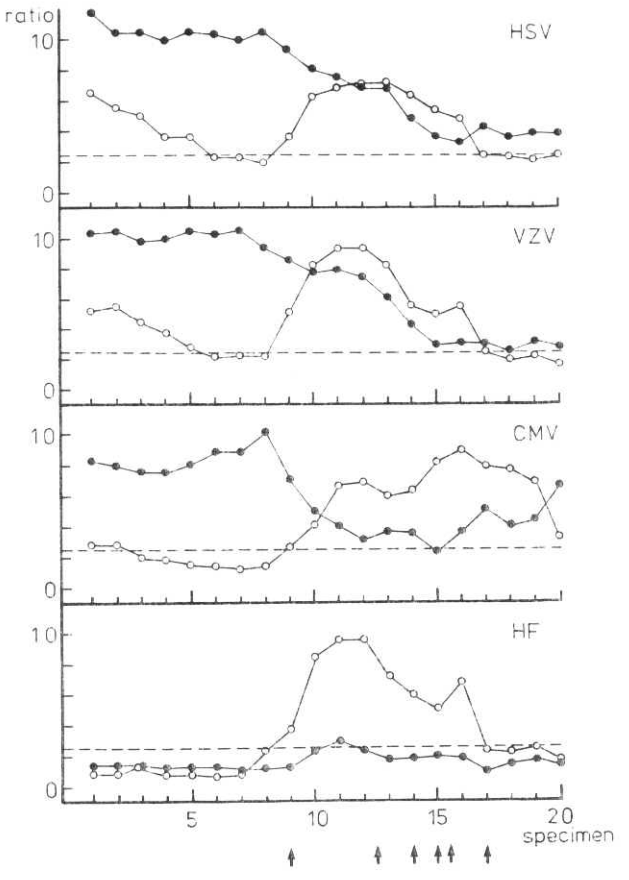

Fig. 1. - Immune response to HSV, VZV, CMV, and human fibroblast antigens of patient No. 1 (Table 2) after bone marrow transplantation.
Solid symbols = IgG antibodies, open symbols = IgM antibodies.
Dashed line indicates positive/negative cutoff line.
Arrows show isolation of CMV from urine and/or throat

Discussion

Serum specimens collected from bone marrow recipients were tested for the presence of IgG and IgM class antibodies against HSV, VZV, CMV and HF antigens. The study was conducted in order to evaluate the value of virus serology in the diagnosis of herpes virus infections in heavily immunosupressed patients. The sera were tested by enzyme immunoassays using lysate antigens of infected and uninfected cells. These crude antigens have been chosen because antigens of similar quality and purity can be supplied by commercial companies. Purified virus antigens can be obtained in very limited amounts and are therefore not widely available for routine diagnostic purposes.

To simplify the test, sera were examined at one single dilution, and the results were compared with the result obtained from a negative reference serum.

Five out of six patients developed significant levels of IgM antibodies to HSV, one patient having a vesicular rash for which no virological etiology could be established. Only from two patients HSV could be isolated during the time of IgM antibody synthesis. It is interesting to notice that the antigen used was able to detect an IgM antibody response during both, HSV-1 and HSV-2 infection.

IgM antibodies against CMV and against VZV were found in two patients. One patient produced IgM to VZV at very low levels simultaneously to the production of high levels of HSV IgM and IgG antibodies during a recurrence of genital herpes. Also

low levels of CMV IgM antibodies were found in sera of this patient in the absence of virus excretion.

One patient showed a strong positive result in IgM assays against all three virus antigens. However, these antibodies were overlapped by high amounts of IgM antibodies to human fibroblast antigens. Without performing absorption experiments it cannot be excluded that part of the IgM antibodies were virus specific. But the patient suffered during this period from a chronic graft versus host reaction, and it may be speculated that these IgM antibodies to HF are an indicator for this reaction. During the same period the patient was repeatedly excreting CMV. The synthesis of CMV IgM antibodies continued for several weeks after IgM to HF had declined to undetectable levels. Simultaneously an increase of CMV IgG antibodies was noticed.

Levels of IgG antibodies in sera of bone marrow recipients are greatly influenced by the graft function, by the degree of immunosupression and by the number of blood transfusions given to the patient. Fluctuations of IgG antibodies may therefore not be indicative for a recent infection with the corresponding virus.

Even if part of the results presented here retrospectively give evidence for some infections caused by herpes viruses, under routine conditions it would be extremely difficult to make a conclusive interpretation of the serological findings upon initial testing of each serum specimen. The number of patients included in this study, and the incidence of virologically proven herpes virus infections during the time of observation are definitely too small to make final conclusions about the usefulness of virus serology in immunosupressed hosts. However, the results indicate that serological values must be interpreted very carefully and that adequate controls have to be included in each test.

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