Aspergillus fumigatus Contamination of Lymphokine-Activated Killer Cells Infused into Cancer Patients

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Lymphokine-activated killer (LAK) cells, prepared by incubating autologous lymphocytes in cell culture medium with interleukin-2, selectively lyse tumor cells and are effective immunotherapy of some cancers. During a 3-month period, two patients at our center were infused with LAK cells subsequently found to have been contaminated by Aspergillus fumigatus. Each case was investigated by obtaining environmental cultures and assessing aseptic practices during LAK cell preparation. Investigation of the first case demonstrated a malfunction of the laminar air flow hood, under which interleukin-2 and the patient's lymphocytes had been added to cell culture medium, and showed heavy A. fumigatus contamination of the hood, adjacent counterops, and cell culture incubator. Despite repair of the laminar air flow hood and cleaning of the laboratory, a second case occurred, and cultures at that time implicated the humidified cell culture incubators as the source of A. fumigatus. Following incubator sterilization and removal of the humidification apparatus from the incubators, weekly environmental cultures in the LAK cell laboratory were negative, and none of the LAK cell cultures from the 20 patients treated during the ensuing 15 months grew A. fumigatus. Our findings show that growth of fungi in humidified incubators, which previously has caused contamination problems in tissue culture and clinical microbiology laboratories, can result in patient infections when humidified incubators are used to prepare cells for reinfusion.

CASE REPORTS

Patient 1. A 25-year-old woman with metastatic melanoma received her first infusion of LAK cells on 10 June 1988. The infusion was halted after about 30 min when she complained of tongue swelling, nasal congestion, and itching of her eyes and ears. As part of the evaluation of her reaction, the remaining infusate (40 ml of an initial 200-ml suspension) was cultured on Columbia agar with 5% sheep blood, chocolate agar, Sabouraud dextrose agar, and brain heart infusion agar. A. fumigatus grew on each of the four agar plates and from a 1-ml sample of the LAK cell suspension collected and cultured routinely in tryptic soy broth 2 h before infusion. Cultures of the patient's mononuclear cells collected on 6 and 7 June before lymphokine activation had been sterile. Despite intravenous infusion of an estimated \(5 \times 10^9\) CFU of A. fumigatus in the contaminated LAK cell suspension (the estimate was based on the growth on agar media of \(\sim 30\) CFU/ml), the patient did not develop clinical evidence of invasive aspergillosis. Nonetheless, beginning 1 week after the infusion of contaminated LAK cells, she received a total of 2.0 g of intravenous amphotericin B over 6 weeks. Serum samples obtained 11 and 11 weeks after infusion of the contaminated LAK cell suspension were tested by Thomas Patterson at Yale University School of Medicine for Aspergillus antigen and antibodies (8). Neither was detected in the initial sample, and antibody alone was detected in the later sample. The patient died 8 months after receiving the contaminated LAK cell suspension, and no evidence of Aspergillus infection was found at the autopsy.

Patient 2. A 28-year-old woman with metastatic melanoma received an infusion of LAK cells uneventfully on 25 August 1988. One-milliliter samples of the cells had been obtained for routine surveillance cultures twice during the 24 h before infusion and inoculated in tryptic soy broth. Two days after infusion of the cells, both broth cultures were noted to be growing a mold subsequently identified as A. fumigatus. Routine cultures of the cells obtained on 23 August, 1 day after the start of lymphokine activation, had been sterile. The patient remained afebrile but 2 weeks later was treated presumptively with amphotericin B. After a total dose of 500 mg, amphotericin B was halted because of azotemia, and therapy was completed with itraconazole at a dose of 200 mg/day for 2 weeks. The patient died in January 1989.

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without clinical evidence of invasive aspergillosis. Serum samples obtained 7 days before and 8 days after the contaminated infusion were negative for _Aspergillus_ antibodies. _Aspergillus_ antigen at a concentration of about 20 ng/ml was detected in the postinfusion sample.

**MATERIALS AND METHODS**

Results of routine cultures of LAK cells infused into other patients during the same period as the contaminated cases were reviewed, and manufacturers of the products used to prepare LAK cells were contacted to see whether product contamination had been reported. Procedures for the collection, incubation, and reinfusion of LAK cells were reviewed after each incident, and environmental cultures were taken in the leukapheresis clinic and LAK cell laboratory. Surfaces were sampled with sterile cotton-tipped swabs premoistened with sterile saline, and air was sampled with a two-stage cascade impactor (Andersen Air Samplers, Atlanta, Ga.). Surface and air samples were inoculated onto Sabouraud dextrose agar (Difco Laboratories, Detroit, Mich.) and incubated at 35°C; colonies of _A. fumigatus_ were identified by standard criteria (9a).

Growth of _A. fumigatus_ was tested in the complete cell culture medium (Whittaker Bioproducts, Inc., Walkersville, Md.) that was used to incubate mononuclear leukocytes from patients 1 and 2. For comparison, growth also was tested in a nutrient fungal medium, Czapek medium (Difco Laboratories). To prepare a starting inoculum, the strain of _A. fumigatus_ from patient 1 was plated on Sabouraud dextrose agar, and a fresh colony was suspended in 10 ml of sterile water. Then 0.5 ml of the suspension was diluted 1:100, and 0.5-ml portions of the dilute suspension were inoculated on Sabouraud dextrose agar for a plate count and into cell culture bags containing 500 ml of Czapek medium or complete cell culture medium. The bags were incubated at 37°C, and 0.5-ml samples were withdrawn from each bag twice daily and plated undiluted or at dilutions up to 1:1,000 onto Sabouraud agar for incubation and colony count.

**RESULTS**

Investigation of patient 1. When the first episode of LAK cell contamination was recognized, we reviewed records of the 30 other patients who had been treated since the inception of the program in September 1987. Aspirates from bags of LAK cells infused into three other patients, all treated in May or June 1988, had yielded _A. fumigatus_. In each case, the culture-positive LAK cell aspirate had been obtained and inoculated into tryptic soy broth in the LAK cell laboratory. Positive cultures in all three patients were considered to represent specimen contamination in the LAK cell laboratory, because subsequent samples of the same cell suspensions submitted to the clinical microbiology laboratory were culture negative. None of the three patients who received the cell suspensions developed aspergillosis.

Manufacturers of the 24 products used to prepare LAK cells for patient 1 were contacted, and all denied receiving reports of contamination problems associated with their products.

Technicians who prepared the LAK cells for patient 1 reportedly followed a standard protocol described by Eberlairn et al. (4). In brief, mononuclear cells were collected in the leukapheresis clinic by using a continuous-flow cell separator and closed-system density gradient centrifugation. Cells were carried in sealed transfer packs to the LAK cell laboratory, where under a laminar air flow (LAF) hood an automated pump was used to transfer the cells and complete medium containing reconstituted IL-2 into 1-liter plastic culture bags which were heat sealed when full. During the 1- to 2-h admixture process, bottles containing complete medium with IL-2 were left open under the hood. The culture bags were placed in either of two humidified incubators and maintained at 37°C for 3 or 4 days before cell separation and reinfusion. One-milliliter aspirates of cell suspension routinely were obtained for culture following initial cell collection, 24 h before reinfusion, and 2 h before reinfusion.

Procedures for LAK cell preparation were observed, and several problems involving the LAF hood were noted. Debris and spilled medium had accumulated under the grill, the interior of the LAF hood was not routinely cleaned before use, and the UV light was not working. Also, testing of the hood revealed that a recently installed room air conditioner created an air current strong enough to interrupt the LAF under the hood.

Cultures of air and surfaces in the leukapheresis clinic and LAK cell laboratory were obtained immediately after the first case of LAK cell contamination was recognized. Specimens which yielded _A. fumigatus_ are summarized in Table 1. Air samples from the leukapheresis clinic and the air supply ducts to the LAK cell laboratory were negative, as were swab cultures of the leukapheresis machine.

On the basis of the above findings, it was concluded that the most likely source of _A. fumigatus_ was the inside of the LAF hood and that contamination of the LAK cell preparation for patient 1 probably had occurred during admixture of mononuclear cells, IL-2, and complete medium under the hood. Contamination of culture specimens from three other patients was attributed to flawed aseptic technique during inoculation into tryptic soy broth. Recovery of _A. fumigatus_ from the inside of an incubator was considered to be a consequence of dispersal of _Aspergillus_ throughout the laboratory.

**Remedial action.** Surfaces and equipment in the LAK cell laboratory, including the incubators, were cleaned and wiped with a 0.5% solution of sodium hypochlorite. The LAF hood was cleaned and serviced, and the technicians who used the hood were reinstructed in aseptic practices during LAK cell preparation and culturing. Complete medium for cell culture was purchased in 10-liter bags, to which tubing from the solution transfer pump could be attached to maintain a closed system. Air samples were obtained from several areas in the LAK cell laboratory on 29 July, after the laboratory and equipment had been cleaned. No colonies of _Aspergillus_ were recovered, so the laboratory was reopened in August. While the above measures were being carried out, another laboratory was used for cell preparation by LAK cell laboratory personnel. Four patients were treated uneventfully with LAK cells prepared in the alternate laboratory.

Investigation of patient 2. The first LAK cells prepared in the reopened laboratory were infused into patient 2. When it was learned that the cells were contaminated with _A. fumigatus_, another investigation was conducted. Cultures obtained during this investigation showed _A. fumigatus_ contamination of both incubators used for LAK cell culturing and heavy growth of _A. fumigatus_ in the water pan placed on the bottom shelf of one of the two incubators (Table 1). The water pan in the second incubator was not cultured. On inspection, dried spilled medium was noted on the shelves of both incubators.

After this episode, the LAK cell laboratory was recleaned,
the incubators were sterilized, the humidification pans in the incubators were removed, and prompt cleanup of spills in the incubators was instituted. In the ensuing 15 months, during which the incubators were operated without humidification, weekly environmental cultures in the laboratory were negative, and none of the LAK cell cultures from 20 patients was positive.

**Growth studies.** Growth of *A. fumigatus* in the complete medium used for activation of mononuclear cells from patients 1 and 2 occurred as rapidly as in a nutrient medium for fungi (Fig. 1). An initial inoculum of about 1 CFU of *A. fumigatus* per 100 ml of medium reached a concentration of >10⁶ CFU/ml in 72 h.

**DISCUSSION**

*A. fumigatus* contamination of LAK cell preparations administered to the two patients described in this report was a significant clinical problem. Each patient was given a lengthy course of antifungal therapy, and LAK cell treatment of other patients enrolled in the same protocol at our center was temporarily halted while the source of contamination was sought. Antifungal treatment was administered presumptively based in part on a previous survey of infectious diseases specialists which found that most would give antifungal therapy following intravenous infusion of a pathogenic fungus into an immunocompromised patient (3). In retrospect, the decision also is supported by the positive results of serologic tests for *A. fumigatus* in the two patients (8, 9) and by recent evidence of IL-2-induced impairment of neutrophil chemotaxis (7).

Our investigation eventually established that the primary source of *A. fumigatus* in the LAK cell laboratory was the humidified cell culture incubators. These incubators were found to be heavily contaminated during the investigation of patient 1, even though attention was focused on the LAF hood as the likely immediate problem. The humidified incubators were the only apparent source of *A. fumigatus* for the second case. The humidified incubators provided conditions highly favorable for the growth of *A. fumigatus*, i.e., a temperature of 37°C and high humidity (1). In addition, nutrients were available from complete medium spilled onto shelves and into the water pans. Incubator contamination by *Aspergillus* species occasionally has been reported in tissue culture laboratories (2, 6) and is part of the oral lore of many clinical microbiology laboratories. Perhaps because published reports of this problem are infrequent, the instruction manuals for the incubators used in our LAK cell laboratory and for similar incubators from other manufacturers did not address the possibility of fungal contamination. Wider awareness of this problem appears especially important because contamination can lead to patient infections if the incubators are used to prepare products for infusion.

The means by which *A. fumigatus* from incubators contaminated LAK cell preparations for patients 1 and 2 could not be ascertained retrospectively. However, it appears that dispersal of *A. fumigatus* from the incubators caused widespread contamination of the LAK cell laboratory, probably by May 1988, when the first false-positive culture was obtained. The LAF hood was heavily contaminated by 14 June, and we speculate that conidia were introduced into LAK cells for patient 1 on 7 June via bottles of complete medium which were left open under the hood for more than

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**TABLE 1. Recovery of *A. fumigatus* from the LAK cell laboratory**

<table>
<thead>
<tr>
<th>Patient (investigation dates)</th>
<th>Specimen</th>
<th>No. of air* or surface samples</th>
<th>No. of CFU of <em>A. fumigatus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1 (14-16 June 1988)</td>
<td>Room air</td>
<td>2</td>
<td>71</td>
</tr>
<tr>
<td></td>
<td>Air under LAF hood (blower off)</td>
<td>1</td>
<td>&gt;200</td>
</tr>
<tr>
<td></td>
<td>Air under LAF hood (blower on)</td>
<td>1</td>
<td>&gt;200</td>
</tr>
<tr>
<td></td>
<td>Inside surface of LAF hood</td>
<td>1</td>
<td>&gt;200</td>
</tr>
<tr>
<td></td>
<td>Outside surface of LAF hood</td>
<td>1</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>Countertop adjacent to LAF</td>
<td>1</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>Inside surface of incubator</td>
<td>1</td>
<td>&gt;200</td>
</tr>
<tr>
<td>Patient 2 (30 August 1988)</td>
<td>Room air</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Air under LAF hood (blower off)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Air under LAF hood (blower on)</td>
<td>1</td>
<td>9*</td>
</tr>
<tr>
<td></td>
<td>Inside surface of LAF hood</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Outside surface of LAF hood</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Inside of cell culture incubator A</td>
<td>1</td>
<td>&gt;200</td>
</tr>
<tr>
<td></td>
<td>Inside of cell culture incubator B</td>
<td>1</td>
<td>&gt;200</td>
</tr>
<tr>
<td></td>
<td>Humidification water in incubator B</td>
<td>1</td>
<td>&gt;200</td>
</tr>
</tbody>
</table>

* Each air sample was 0.57 m³ (20 ft³).

* The cell culture incubators were opened for inspection and culturing while this air sample was being obtained.
1 h during the addition of mononuclear cells and IL-2 to the medium. The mechanism of contamination of LAK cells infused into patient 2 appears to have been different because of the change to a closed solution transfer system and decontamination of the LAF hood. Also, in view of the rapid growth of *A. fumigatus* in cell culture medium, the negative culture obtained 1 day after the start of incubation strongly suggests that contamination had not yet occurred. Possible mechanisms include faulty aseptic technique when aspirates for culture or cell count were taken from the incubating suspension and introduction of organisms through inapparent microleaks in the cell culture bag. Authors of previous reports of mold contamination of solutions for intravenous infusion generally have attributed the problem to hairline cracks in glass containers (3, 10) or other physical factors (13), even when manipulation under a LAF hood may have been performed periodically. Incubators should be used.

In view of the efficacy of IL-2 and LAK cells in initial studies, use of these therapies by an increasing number of centers can be anticipated. The need for rigorous infection control measures during preparation of LAK cells is evident from the events described in this report. On the basis of our experience, we suggest the following: (i) at the outset, procedures for LAK cell preparation should be critically reviewed by personnel trained in infection control and clinical microbiology laboratory practices, (ii) personnel who prepare LAK cells should undergo specific training in laboratory asepsis, (iii) LAF hoods should be inspected periodically and after any modification of the ventilation system in the laboratory, (iv) even minor problems, such as false-positive surveillance cultures, should prompt a thorough investigation, and (v) humidified incubators should be viewed as potential hazards for fungal growth, and whenever feasible, dry incubators should be used. If high humidity must be maintained, surveillance cultures of incubators should be performed periodically, incubators should be cleaned frequently, and addition of antifungal agents to the humidification water should be considered.

REFERENCES