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TREATMENT OF VISCERAL LEISHMANIASIS IN 2004

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Abstract. In 2004, visceral leishmaniasis (kala-azar) maintains its status as a neglected, if not "most neglected" disease. Lack of affordable new drugs, still a basic unsolved problem, has also been joined by additional therapeutic obstacles including large-scale resistance to pentavalent antimony (Sb) in India and coinfection with human immunodeficiency virus in all endemic regions. Nevertheless, available treatment options have actually expanded because the energetic clinical trials effort of the past decade has yielded tangible advances. This progress includes successful application of less expensive generic Sb; rediscovery of the high-level efficacy of amphotericin B; implementation of short-course parenteral regimens (lipid formulations of amphotericin B); potential to replace Sb and amphotericin B with price-capped paromyomycin; and identification of the first effective oral agent (miltefosine). How to sustain and move this progress ahead, make new advances practical (e.g., affordable, and therefore, deployable), and how to translate promising experimental approaches into actual therapy remain difficult next steps in the treatment of kala-azar.

Nearly coincident with the 100th anniversary of the original clinical descriptions of visceral leishmaniasis (VL) (kala-azar) (by Leishman and Donovan in 1903), the status of this disseminated intracellular protozoal infection was upgraded. Regrettably, the upgrade was to that of a "most neglected" disease,[1] a sobering promotion for kala-azar from its traditional position as simply a "neglected" disease. The new designation is not particularly surprising, however, since except for endemic regions in southern Europe, kala-azar remains for the most part firmly embedded in high-level poverty. Approximately 90% of the estimated 500,000 new annual cases of visceral disease occur in rural areas of India, Nepal, Bangladesh, Sudan, and Brazil in some of the world's poorest regions (Figure 1). Predictably, then, practical advances in prevention and control and in diagnosis and proper treatment remain difficult to develop and/or implement in the countries where such advances are needed most. In this way, VL is not different from other infectious diseases similarly trapped by poverty.

Nevertheless and even though traditional and newly-inserted roadblocks still stand squarely in the way in 2004, laboratory and clinical research are being translated in kala-azar and tangible progress has been made.[2–4] Thus, without at all diminishing the urgency of developing vaccine, devising better vector control, applying non-invasive diagnosis, and ensuring drug supply and distribution,[2,3] it is also fair to point out that VL may not actually fulfill all of the typical criteria of a most neglected disease.[1] Without doubt, the lack of truly affordable new drugs remains a basic, unsolved problem. However, on the other side of the ledger, the efforts in chemotherapy of the past 10 years have clearly led to expansion rather than contraction of available options (Table 1),[4,5] which now include both highly-active short-course parenteral regimens and effective oral therapy. This report highlights the recent progress made in the treatment of VL.

TREATMENT IN 1990

To put new developments into perspective, it is useful to recall that in 1990 there was essentially only one form of treatment of visceral infection, one of the pentavalent antimony (Sb) preparations. These agents, meglumine antimoniate (Glucantime®; Aventis, Strasbourg, France) and sodium stibogluconate in branded (Pentostam®; GlaxoSmithKline, Uxbridge, United Kingdom) or generic form, showed time-tested worldwide efficacy (> 90% cure in 1990), but required daily injections for up to 28 days and produced well-recognized toxicity. For the increasing numbers of patients (primarily in India) who failed initial Sb treatment in the early 1990s, prolonged re-treatment with Sb, switching to pentamidine, or combining these two agents were the alternatives. These salvage approaches were initially but not ultimately useful because of extraordinary treatment duration, toxicity and/or flagging efficacy.[4,5]

TREATMENT IN 2004

If one fast-forwards to 2004, the therapeutic landscape in kala-azar has clearly changed (Table 1). Between 1990 and 2003, more than 60 treatment trials were carried out in India, Brazil, southern Europe, Sudan, and east Africa. This effort and its success in generating new treatment alternatives and/or therapeutic refinements can be traced back to at least four factors that converged in the mid 1990s: 1) increasing Sb resistance in Bihar State, India (which houses contains approximately 40% of the world's cases) with no clear-cut substitute; 2) initial therapeutic successes upon which to build using both old and new drugs alone and in combination (amphotericin B, aminosidine, lipid formulations of amphotericin B, Sb plus aminosidine); 3) rekindled efforts by international agencies; and 4) the entry of new clinical investigators into the field focused on improving treatment and testing new agents (Figure 2).[2,4] These same factors continued to drive the progress, yielding further tangible advances: short-course therapy (lipid formulations of amphotericin B), more affordable treatments (generic Sb, paromyomycin [aminosidine]), and oral therapy (miltefosine). Table 2 summarizes illustrative treatment protocols for each of the preceding agents.

PARENTERAL AGENTS: CURRENT STATUS AND NEW DEVELOPMENTS

Pentavalent antimony (Sb). Even after more than 50 years of continuous use, Sb in branded or generic form remains conventional treatment of VL in children and adults in all regions, with two exceptions. First, in Bihar State, which contains approximately 90% of the estimated 200,000–250,000 annual new cases in India, Sb is no longer useful; failure rates, defined as initial unresponsiveness or prompt post-treatment

FIGURE 1. **Left**, Typical village setting in which kala-azar is acquired in Bihar State, India (Muzaffarpur district, the epicenter of the ongoing Indian epidemic). **Right**, Patient being carried to a visceral leishmaniasis treatment unit in Duar, Sudan (photograph provided by Dr. Barbara Herwaldt). This figure appears in color at www.ajtmh.org.

TABLE 1
Treatment alternatives and approaches for visceral leishmaniasis: 1990 versus 2004[4,5]

Approach and agents*	1990	2004	Selected comments related to past decade's experience, 1994–2004
Parenteral agents used alone			
Pentavalent antimony (Sb)	+	+	Field efficacy reconfirmed in Sudan epidemic, but resistance ended usefulness in Indian epidemic (Bihar State). Generic Sb active in east Africa.
Pentamidine	+	+	No reason to use.
Amphotericin B deoxycholate		+	Rediscovered as Sb resistance increased in India.
Lipid formulations of amphotericin B AmBisome, Abelcet, Amphotec (Amphocil)		+	Highly active, efficient treatments worldwide but prohibitively expensive.
Paromyomycin (aminosidine)		+	Active as single agent in India, including in Sb failures. Being retested now in India as potential replacement for Sb and/or amphotericin B.
Combination parenteral agents			
Sb plus paromyomycin		+	First combination tested to enhance efficacy and/or reduce treatment duration.
Immunochemotherapy			
IFN-γ plus Sb		+	Possibly useful as re-treatment in Sb failures (if parasite not Sb resistant)
GM-CSF plus Sb		+	Possibly useful in ameliorating leukopenia-related secondary infections.
Oral agents alone and in combination			
Miltefosine		+	First highly effective oral therapy for kala-azar (India).
Sitamaquine		+	Limited studies (Brazil, Kenya) suggest promise. Trial results from India pending.

* Except for granulocytes–macrophage colony-stimulating factor (GM-CSF) plus Sb (Brazil), treatments listed have been tested in at least three or more trials. Since 1990, additional pilot studies or single trials in India have reported 1) apparent efficacy for amphotericin B deoxycholate hand-mixed with a lipid emulsion, generic liposomal amphotericin B, and low-dose pentamidine plus allopurinol, and 2) limited efficacy for fluconazole used alone or in combination with atovaquone.[4] IFN-γ = interferon-γ.

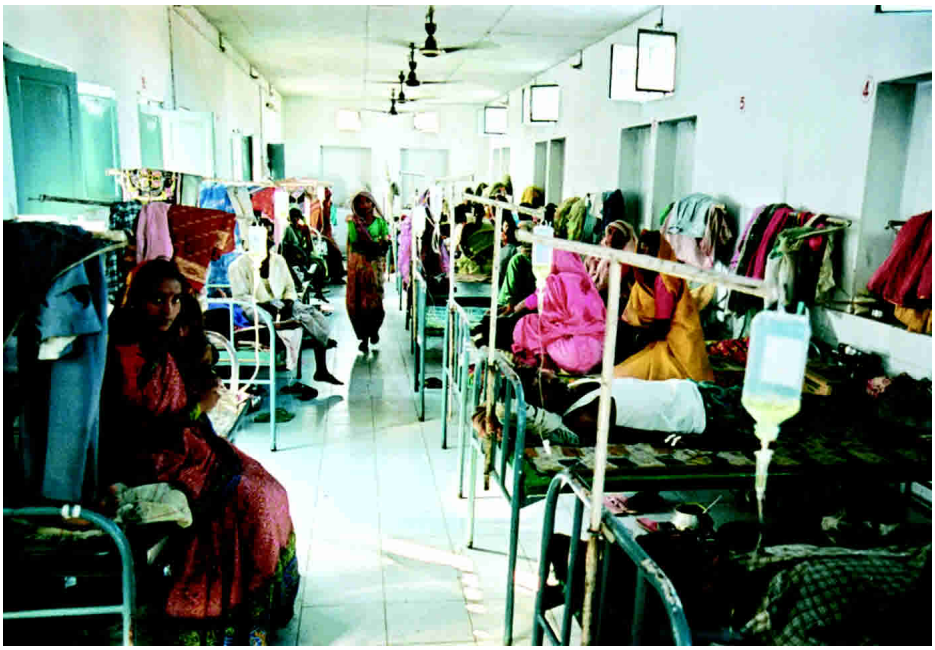

FIGURE 2. Inpatient ward at Kala-Azar Medical Research Center, Muzaffarpur, Bihar State, India, established by Dr. Shyam Sundar (who provided the photograph). Patients are receiving infusions of amphotericin B in one of many clinical trials directed by Dr. Sundar at this referral center. This figure appears in color at www.ajtmh.org.

relapse, reached 65% more than five years ago.[6] While Sb is currently effective in other areas of India and surrounding countries, clinicians in Bihar necessarily turned to the only then-available, realistic (e.g., "affordable") alternative, amphotericin B deoxycholate (Fungizone; Bristol-Myers Squibb, New York, NY).

Second, Sb use in Mediterranean kala-azar has also decreased considerably, not because of drug resistance, but because of increasing deployment of the highly effective and efficient lipid formulations of amphotericin B. Clinicians in southern Europe have tested and primarily use liposomal amphotericin B (AmBisome®; Gilead Sciences, Foster City, CA);[7,8] however, amphotericin B cholesteryl sulfate (Amphotec® [Amphocil™]; InterMune Corp., Burlingame, CA) and amphotericin B lipid complex (Abelcet®; Enzon Pharmaceuticals, Bridgewater, NJ) are also available and likely just as effective.[4,5] The remarkable cost of these agents limits their application even in southern Europe; nevertheless, AmBisome has replaced Sb in Italy[8] and use in other Mediterranean countries has increased predictably as well.

The one recent positive development in the use of Sb in VL relates to thorough testing of a particular generic preparation (Sodium Stibogluconate Injection; Albert David, Ltd., Calcutta, India) provided via the International Dispensary Association (Amsterdam, The Netherlands).[9] In a series of comparative studies carried out in Kenya, Ethiopia, and Sudan,

TABLE 2
Representative treatment regimens for visceral leishmaniasis in immunocompetent patients[4,5]

Agent	Dose	Schedule	Cure (%)*	Region (in use or tested) and comments
Injectible therapy				
Pentavalent antimony (Sb)	20 mg/kg	Daily × 28–30 days	~90	Active worldwide except in Bihar State, India.[6]
Amphotericin B deoxycholate	1 mg/kg	15 doses over 30 days	~95	India. First-line parenteral therapy in Bihar.[4,10]
		or daily × 20 days	>95	Alternate-day regimen presumed less toxic.
Lipid amphotericin B				
AmBisome	3 mg/kg	Daily × 5 days + dose 6 on day 10	~95	Southern Europe (in wide use).[7,8,21]
	1–2 mg/kg	Daily × 5 days	93–96	India (tested).[11,15,16]
Abelcet	2 mg/kg	Daily × 5 days	92	India (tested).[5,11]
Amphotec (Amphocil)	2 mg/kg	Daily × 7 days	100	Brazil (limited testing).[13]
	2 mg/kg	Daily × 7 days	100	Italy (limited tested).[14]
	Escalating	7.5 mg/kg (total) over 6 days	97	India (tested).[11]
Paromyomycin	16–20 mg/kg	Daily × 21 days	93–97	India (tested).[19] Phase III trial (India, 15 mg/kg/day × 21 days) in progress.
Oral therapy				
Miltefosine	50 mg	Twice (≥ 25 kg) or once a day (< 25 kg) × 28 days†	~95	India (in use).[4,20] Phase IV study (India) pending.

* Healthy with no signs or symptoms of relapse six months after treatment.
† (≥ 25 kg) and (< 25 kg) indicates body weight.

generic Sb was safe, well-tolerated, and as effective as Pentostam at a fraction (1/8) of the cost of the latter per treatment course (approximately US $22 versus US $180–200).[9] Other generic forms of Sb, manufactured and used in India, China, and Brazil, have not been well-tested in other countries.

Amphotericin B deoxycholate. Since resistance to Sb has not yet meaningfully surfaced in other regions, virtually all of the published experience with amphotericin B has been generated in India (Bihar State).[4,5] In short, amphotericin B is effective, but arduous treatment with drawbacks that include well-known adverse reactions and toxicity, the requirement for infusions, prolonged duration of therapy (up to 30 days, Table 2) and comparatively high overall cost of treatment. Nevertheless, in Bihar, amphotericin B is currently first-line parenteral therapy, and regularly induces long-term cure rates $\geq$ 95%.[4,5,10]

Lipid formulations of amphotericin B. The physicochemical nature of these agents enables preferential uptake by tissue macrophages in the liver, spleen, and bone marrow, the cells and organs targeted in VL (Figure 3). These well-tolerated drugs are remarkably active in kala-azar and effective in children and adults in all endemic regions and under diverse field conditions. Provided that sufficient doses are given, cure rates of approximately 95% are nearly routine in patients treated with AmBisome, Abelcet, or Amphotec, including those who receive short-course regimens.[4,5]

AmBisome is the only Food and Drug Administration-approved lipid preparation for treatment of VL and has been most widely tested. Results from a recent three-arm study in India demonstrated that 1) AmBisome and Abelcet (each given at a dose of 2 mg/kg/day for 5 days) produced far fewer infusion-related reactions versus conventional amphotericin B (15 alternate day 1 mg/kg infusions over a 30-day period) and little of the other toxicity of the latter drug (e.g., renal insufficiency, hypokalemia, anemia); 2) AmBisome induced significantly fewer infusion reactions and more prompt defervescence versus Abelcet; and 3) overall cure rates appeared similar (amphotericin B = 96%, AmBisome = 96%, Abelcet = 92%).[11]

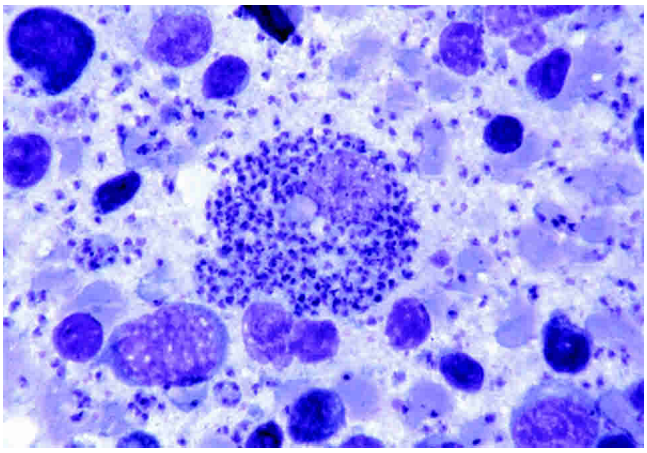

FIGURE 3. Giemsa-stained splenic aspirate smear (provided by Dr. Roberto Badaro) showing heavily-parasitized macrophages and numerous *L. chagasi* amastigotes in a Brazilian child with a visceral infection (magnification × 200). This figure appears in color at www.ajtmh.org.

Findings in a tri-continental AmBisome study have suggested regional variations in clinical and parasitologic responsiveness in patients with VL: total doses required for 100% cure were low in India (6 mg/kg, *Leishmania donovani*), higher in Kenya (14 mg/kg, *L. donovani*), and highest in Brazil (> 20 mg/kg, *L. chagasi*).[12] Similarly high total doses of AmBisome (18–20 mg/kg) are also needed in the Mediterranean region (*L. infantum* [identical to *L. chagasi*]).[2,7,8] Why Brazilian and Mediterranean kala-azar require higher-dose therapy is not clear, but this may reflect the infecting species and/or the primary population targeted (young children). Alternatively, Indian kala-azar, which develops primarily in adults and older children, may simply be more treatment-responsive in general.

Abelcet and Amphotec have also been tested in VL with similar success: Abelcet thoroughly in India but not elsewhere[5,11] and Amphotec in limited studies in Brazil and Italy.[13,14] Recent preliminary data from India also indicate that Amphotec induces high-level cure rates in Bihar State as well (Table 2).[11]

The particular usefulness of the lipid formulations in VL, however, lies in the non-toxic, macrophage-targeted delivery of sufficient amphotericin B to induce high cure rates after relatively few doses. Thus, when used at 3–5 mg/kg (or even up to 10–15 mg/kg) per infusion, these agents have made clinically appealing short-course regimens a reality.[4] Cure rates of 90–100%, which depend upon the total doses of the preparation administered,[4] can be achieved by once a day infusions for five days in India[11,15] or by six or seven infusions given over a 7–10-day period in Brazil and southern Europe (Table 2).[7,8,13,14] Using AmBisome, which seems best-suited pharmacologically for ultra-short-course therapy, the treatment duration can apparently be compressed still further: to a single day in India in adults and children (one infusion of 5 or 7.5 mg/kg)[16,17] and to two days in children in Greece (two infusions of 10 mg/kg each)[18] (Table 3). In both instances, the total dose of AmBisome, which would have been administered over a 5- or 10-day period in India or Greece, respectively, was given in one or two infusions.

Nonetheless and despite clinical enthusiasm, the strikingly high cost of AmBisome, Abelcet, and Amphotec (approximately $US 160–175 or more per 50 mg) has put even short-course regimens beyond the reach of most patients with VL. The one regional exception is southern Europe, the only endemic area where cost alone is not the primary determinant of whether any antileishmanial treatment, irrespective of efficacy and/or efficiency, is deployed. The frustrating obstacle of drug cost and efforts to reduce such costs have been reviewed in detail elsewhere,[4] and are considered again later in this report.

Paromyomycin. This aminoglycoside (identical to aminosidine) was tested alone and in combination with Sb in kala-azar patients in Kenya, India, and Sudan in the early 1990s.[4] More recently, paromyomycin given by itself for 21 days demonstrated good activity in India (approximately 95% cure), including in patients who had failed prior Sb treatment.[19] International efforts have successfully resurrected a newly manufactured preparation that is now being tested in India (once a day intramuscular injections of 15 mg/kg for 21 days). Assuming that its high-level efficacy and low rate of adverse reactions are redocumented, the one drawback of paromyomycin (prolonged treatment duration) should be satisfactorily bal-

TABLE 3
Trials using ultra-short course treatment for visceral leishmaniasis

Agent and dose	No. of doses	Total dose (mg/kg)	No. of patients	% Cure*	Country tested	Cost of drug	(US$)† Total	Comments
Abelcet (mg/kg)								
5	1	5	27	70	India	438	460	Low cure rate.[5]
5	2	10	50	80	India	875	952	Doses on days 1 and 2 or days 1 and 5. Low cure rate.[5]
AmBisome (mg/kg)								
15	1	15	17	100	India	1,200	1,222	Pilot trial. Dose tolerated but extremely high drug cost.
5	1	5	51	91	India	400	422	Efficient (as active as giving same total dose over 5 days [1 mg/kg/day × 5 days]). Total cost equal to amphotericin B (30-day regimen)‡ but efficacy lower than > 95%.[16]
7.5	1	7.5	203	90	India	600	622	Highly efficient (all subjects discharged 24 hours after treatment) but not cost-effective vs. amphotericin B§.[17]
10	2	20	41	98	Greece	1,400	2,080	Doses on days 1 and 2. If body weight < 30 kg, clearly cost-effective vs. 30-day Sb regimen‡ since an ~ 80% reduction in hospital stay (mean = 6.2 days) offsets high drug cost.[18]

* Six months after treatment.
† Per patient costs in India calculated for a 25-kg patient.[4] Per patient costs in Greece extrapolated from those reported for the subjects enrolled in the trial (young children; mean age = 3.6 years).[18] Drug costs: 1) Abelcet = $175/50 mg (U.S. average wholesale price; Abelcet not available in India), and 2) AmBisome = $160/50 mg or $206/50 mg, retail costs in India and Greece, respectively.[4,18] Total cost = drug cost plus all other treatment or hospitalization-related expenses, as described,[4] including hospital per diem charges of ~$2 (India[4]) and ~$110 (Greece[18]).
‡ Estimated per patient cost in India for 15 alternate-day infusions of 1 mg/kg of amphotericin B deoxycholate (drug = $49; total cost = $417).[4]
§ Estimated per patient cost in Greece for once a day injections of 20 mg/kg of Sb for 30 days (drug = $50; total cost = $3,250).[18]

anced by its proposed cost to be capped at US $45.[2] Since the 21-day schedule for this drug is also attractive compared with the 28–30 days for Sb or amphotericin B, paromyomycin has the potential to replace amphotericin B (and residual Sb use) in India, and, if tested successfully elsewhere, could replace Sb in other regions as well.

ORAL AGENTS: CURRENT STATUS

Miltefosine. In contrast to the opaque but apparently ongoing testing of sitamaquine (GlaxoSmithKline), an oral 4-aminoquinoline initially designated as WR6026, the development of miltefosine for treatment of kala-azar was direct and rapid.[4] The first phase I/II study was carried out in India in 1997, and its results opened the door to achieving the long-sought after objective of effective oral therapy. Six additional investigator/company- or WHO/company-initiated trials were completed within three years in India, followed by successful registration in India in 2002 (Impavido®; Zentaris GmbH, Frankfurt, Germany).[4]

Hexadecylphosphocholine (miltefosine) is a membrane-activating alkylphospholipid originally developed as an antineoplastic agent. Initial testing in kala-azar patients, which was based upon solid experimental antileishmanial activity, demonstrated gastrointestinal toxicity but also obvious clinical and parasitologic effects. Succeeding trials, performed in a region of high-level Sb resistance (Bihar State), showed satisfactory safety, tolerable adverse reactions, and efficacy in both adults and children, about one-third of whom had failed Sb treatment.[4] Miltefosine given for 28 days at a dose of 50 mg once or twice a day (depending on a body weight < 25 kg or ≥ 25 kg, respectively) induced cure rates of approximately 90–95% (range = 87–100%). In a large, randomized phase III trial, long-term cure rates were 94% after 28 days of miltefosine therapy (n = 299) versus 97% after amphotericin B treatment (15 alternate-day infusions of 1 mg/kg) (n = 99).[20]

Miltefosine commonly induces anorexia, nausea, vomiting (approximately 60%) and diarrhea (approximately 20%); however, these reactions are typically brief and usually resolve as treatment of VL continues.[4,20] Renal insufficiency and increases in levels of hepatic transaminases are much less frequent and reversible once use of the drug is discontinued. Miltefosine is teratogenic in animals and cannot be used in pregnant women. The drug should also not be used in women of child-bearing age unless a pregnancy test result is negative and adequate contraception can be assured during and for two months after treatment.

One obvious benefit of an active oral agent is treatment outside the hospital. However, the key question of how miltefosine would fare clinically in the unsupervised outpatient setting was necessarily left open, since the trials leading to registration were all performed in carefully selected and monitored inpatient subjects. To generate this important information (e.g., compliance, efficacy of self-administered drug), nearly 1,200 adults and children were enrolled in a recently completed phase IV study in India. After up to three initial in-hospital days for treatment and observation, subjects then completed 28 days of treatment as outpatients. These results will clearly be a relevant guide to the proper use, efficacy, and future clinical impact of this agent in the field.

Since Indian kala-azar may be more treatment-responsive in general, it is important to point out that miltefosine has not yet been tested in VL in other regions. However, studies are now being extended to Nepal and are planned in Brazil, Kenya, and Sudan. In a recently initiated trial in Ethiopia, patients coinfected with human immunodeficiency virus (HIV) are being specifically included because, other than compassionate use in southern Europe, miltefosine has not been tested in acquired immunodeficiency syndrome–related VL. In addition, concern about possible resistance developing in the future has prompted the suggestion to test miltefosine now in combination with a second agent in an effort to protect its current high-level efficacy.[2]

COST OF TREATMENT

In patients with VL in southern Europe, hospitalization and medical care costs are substantial. Thus, virtually any approach that reduces duration of therapy or shifts treat-

ment to the outpatient setting can offset the cost of expensive drugs, even the lipid formulations of amphotericin B. An example of the former approach has been reported in Greek children treated with a two-day, high-dose AmBisome regimen (Table 3).[18] Results in adults in Italy,[21] where AmBisome is administered on five consecutive days with a sixth dose on day 10, have illustrated the latter approach in patients discharged after five doses to receive the day 10 infusion as an outpatient. This simple adjustment was sufficient to render AmBisome treatment cost-neutral, that is, its total cost was equal to that of conventional, in-hospital-administered Sb (21-day course), despite the acquisition price of AmBisome in Naples ($155 per 50 mg).[21]

In direct contrast, since > 90% of cases of kala-azar arise in regions where bare-bones national health expenditures are as little as $5–10 annually per capita, the only truly affordable treatment would be one distributed at essentially no charge. The only drug that even comes close to this likely unattainable goal is generic Sb (approximately $22 per treatment course in an adult).[4] This $22 figure, however, does not include hospitalization and medical care, which still mount up, even in regions such as India with hospital per diem charges of only approximately $2. Assuming Sb therapy was still an option in India, these latter expenses come to approximately $359 for 30 days of treatment in Bihar State.[4]

Largely by default, then, a total cost (drug plus hospitalization) of approximately $375–400 per treatment course has been an unofficial benchmark in kala-azar in poor regions. For comparison (reviewed by Murray;[4] see Table 3), estimated costs in India for selected regimens in Table 2 for a 25-kg adult include amphotericin B, 30-day treatment (drug = $49, total = $417); AmBisome, 2 mg/kg/day for five days (drug = $800, total = $872); and Abelcet, 2 mg/kg/day for five 5 days (drug = $875, total = $947). To bring the total expense of the latter two short-course regimens into line with that of amphotericin B (the current benchmark in Bihar State), the cost of AmBisome and Abelcet would need to be subsidized or substantially reduced (by approximately 60%).[11] Since neither subsidy nor price reduction have materialized, a cost-neutral regimen (total cost equal to that of amphotericin B treatment) of single-dose AmBisome (5 mg/kg) was tested in India and appeared to perform well (91% cure).[16] However, when considered together with the 90% cure rate in a follow-up study (a single dose of 7.5 mg/kg that was not cost-neutral; Table 3),[17] there may well be some trade-off for the high-level efficiency of single-infusion treatment, namely, apparently somewhat lower efficacy (versus > 95% cure expected with conventional amphotericin therapy).

In contrast to the preceding obstacles, price-capping (paromomycin) and eliminating most if not all in-patient care (miltefosine) should further strengthen the appeal of these already appealing agents. Estimated total per patient costs in India (paromyomycin, drug = $45 [projected], total = $285; miltefosine, drug = $100 [proposed], total = $160)[4] should be well below those of amphotericin B deoxycholate and Sb (used outside of Bihar).[6]

TREATMENT IN T CELL-IMPAIRED OR -DEFICIENT PATIENTS

Successful host defense in VL requires T cells, and is likely mediated by inflammatory cytokines (primarily CD4 [Th1 type] cell-derived) that activate macrophages and induce intracellular parasite killing.[4,5] Not surprisingly, then, reactivated (or newly acquired) kala-azar is a recognized opportunistic infection in patients rendered functionally T cell deficient by treatment (corticosteroids, anti-rejection agents in organ transplant recipients) or T cell depleted by advanced HIV disease. Limited data suggest that conventional therapy (Sb, amphotericin B) may produce reasonable results in transplant recipients who survive beyond the first week of hospitalization.[4] However, the experience largely generated in southern Europe (in particular, by Spanish investigators) indicates that the current treatment of HIV-associated VL is not satisfactory.[22,23] In such patients who typically show CD4 cell counts < 200 cells/mm^3 (and often much lower), initial responses to Sb, amphotericin B, or Abelcet are appreciably reduced (e.g., to approximately 40–65%). In addition, adverse drug reactions are frequent, and in clinical responders, relapse rates within 12 months after discontinuing treatment are predictably high (approximately 50–70%).[22,23] In the only other trial from another region (Ethiopia), Sb treatment induced cures in 44% of HIV-coinfected subjects versus 92% of those who were HIV seronegative.[24]

Most of the subjects in the preceding trials were not receiving highly active antiretroviral therapy (HAART) at the time the studies were carried out. Satisfactory increases in CD4 cell counts, especially to > 200 cells/mm^3, likely enhance responsiveness to antileishmanial chemotherapy and are important in preventing relapse. However, while HAART has reduced the overall frequency of VL as an opportunistic infection in southern Europe, it is not entirely clear how well the initial response to antileishmanial treatment is influenced by the extent of HIV suppression, and symptomatic relapse may also occur despite HAART.

A variety of agents, administered as infrequently as once every 2–4 weeks (e.g., Sb, pentamidine, Abelcet, and others) appear capable of reducing post-treatment relapse in coinfected patients.[22,23] Nevertheless, there is no consensus about the regular use of long-term maintenance therapy even in patients clearly at risk for relapse, for example, HAART recipients in whom CD4 cell counts do not increase to and/or stay above 200 cells/mm^3. It is worth pointing out, however, that in some HIV-coinfected patients with quite low CD4 cell counts, persistent visceral infection (documented before and/or after antileishmanial treatment) may be entirely subclinical and remain curiously asymptomatic for extended periods. Such observations, coupled with other factors (decreasing numbers of coinfected patients [at least in Europe] and absence of firm guidance from a large controlled study) will likely continue to frustrate any consensus about optimal post-treatment management in HIV-associated VL.

IMMUNOCHEMOTHERAPY AND OTHER EXPERIMENTAL TREATMENTS

The question of whether experimental treatment approaches in VL (immunotherapy, new chemotherapy) can ever reach clinical application devolves back to the conundrum of being faced with a neglected disease with no real market opportunities to support new drug development.[1] Nevertheless and putting aside these realities, it is also of interest to consider from the therapeutic perspective what is being accomplished in the research laboratory.

Abundant experimental evidence (and supportive clinical observations) indicate that multiple T cell- and macrophage-activating cytokines likely interdigitate to mediate host defense in visceral infection.[4,5] Two of these cytokines, interferon-γ (IFN-γ) and granulocyte–macrophage colony-stimulating factor, graduated nearly 10 years ago to limited clinical testing in combination with Sb (Table 1).[4,5] Since symptomatic VL can be viewed simplistically as a failure in macrophage activation, IFN-γ was used as an adjunct to Sb to directly stimulate tissue macrophages and trigger intracellular leishmanicidal mechanisms[4,5] (Figure 4). The hope was that such a combination, active experimentally, could improve outcome by accelerating the kinetics of parasite killing, reducing the duration of chemotherapy and/or by enhancing overall efficacy. However, results with Sb plus IFN-γ in Brazil, Kenya, and India were mixed, and additional studies have not been pursued. Granulocyte–macrophage colony-stimulating factor also enhances macrophage antileishmanial activity, and readily mobilizes and delivers myelomonocytic cells to infected tissue foci.[5] It was given by Brazilian investigators to ameliorate the leukopenia of kala-azar, and in a single study, this effect appeared to reduce secondary, complicating infections.[25]

Additional experimental approaches to induce or redirect (harness) the efficacy of the T cell-dependent immune response of the host have been identified, but not tested clinically.[4,5] Such approaches, which are active alone and in combination with chemotherapy, have focused on 1) interleukin 12 (IL-12), an IFN-γ-inducing regulatory cytokine that drives the curative Th1 cell-type response, 2) more proximal T cell-antigen-presenting (dendritic) cell events including T cell costimulation via CD40 ligand:CD40 and CD28:B7, and 3) macrophage intracellular signaling mechanisms. An entirely separate approach to immunoenhancement in experimental visceral infection, targeting suppressive down-regulating cytokines such as IL-10 for inhibition, has also proven effective alone and in combination with Sb or amphotericin B.[4]

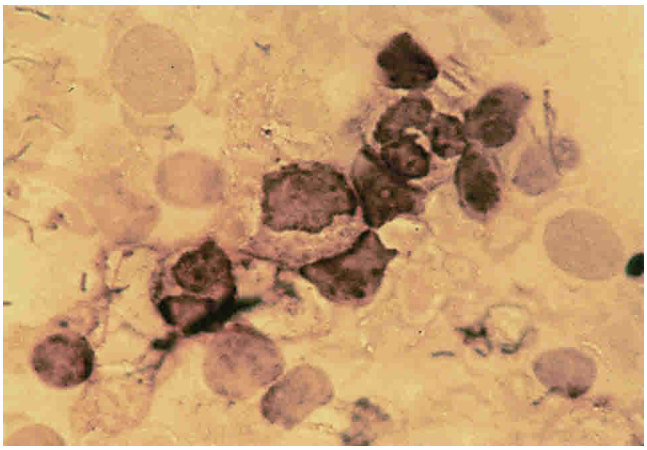

FIGURE 4. Splenic aspirate smear (provided by Dr. Shyam Sundar), stained to detect immunoreactive inducible nitric oxide synthase (iNOS), from an Indian patient with kala-azar. Cells expressing iNOS (**brown reaction product**) are primarily monocytes or macrophages; adjacent lymphocytes show no iNOS staining (magnification × 300). iNOS is induced by activating cytokines (e.g., interferon-γ), and secretion of iNOS-generated reactive nitrogen intermediates represents one macrophage leishmanicidal mechanism.[5] This figure appears in color at www.ajtmh.org.

Experimental efforts specifically directed at developing chemotherapy also continue in laboratories around the world, and the state of this research has recently been reviewed elsewhere.[26] Current approaches include modifying existing drugs, identifying novel compounds (including those rationally selected with validated parasite targets), and improving drug delivery systems by targeting parasitized macrophages. Work directed at heated amphotericin B deoxycholate (heated-induced aggregates are phagocytized by tissue macrophages[26]) and at manipulating Sb to produce an orally-active form[27] are both of particular interest.

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REFERENCES

1. Yamey G, 2002. The world's most neglected diseases. *BMJ 325:* 176–177.
2. Guerin PJ, Olliaro P, Sundar S, Boelaert M, Croft SL, Desjeux P, Wasunna MK, Bryceson ADM, 2002. Visceral leishmaniasis: current status of control, diagnosis, and treatment, and a proposed research and development agenda. *Lancet Infect Dis 2:* 494–501.
3. Murray HW, 2002. Kala-azar - progress against a neglected disease. *N Engl J Med 347:* 1793–1794.
4. Murray HW, 2004. Progress in treatment of a neglected disease: visceral leishmaniasis. *Expert Rev Anti-Infect Ther 2:* 279–292.
5. Murray HW, 2001. Clinical and experimental advances in treatment in visceral leishmaniasis. *Antimicrob Agents Chemother 45:* 2185–2197.
6. Sundar S, More DK, Singh MK, Singh VP, Sharma S, Makharia A, Kumar PCK, Murray HW, 2000. Failure of pentavalent antimony in visceral leishmaniasis in India: report from the center of the Indian epidemic. *Clin Infect Dis 31:* 1104–1106.
7. Gradoni L, Bryceson A, Desjeux P, 1995. Treatment of Mediterranean visceral leishmaniasis. *Bull World Health Organ 73:* 191–197.
8. Gradoni L, Gramiccia M, Scalone A, 2003. Visceral leishmaniasis treatment, Italy. *Emerg Infect Dis 9:* 1617–1620.
9. Veeken H, Ritmeijer K, Seaman J, Davidson R, 2002. A randomized comparison of branded sodium stibogluconate and generic sodium stibogluconate for visceral leishmaniasis under field conditions in Sudan. *Trop Med Int Health 5:* 312–317.
10. Thakur CP, Singh RK, Hassan SM, Kumar R, Narain S, Kumar A, 1999. Amphotericin B deoxycholate treatment of visceral leishmaniasis with newer modes of administration and precautions: a study of 938 cases. *Trans R Soc Trop Med Hyg 93:* 319–323.
11. Sundar S, Mehta H, Sures AV, Singh SP, Rai M, Murray HW, 2004. Amphotericin B treatment for Indian visceral leishmaniasis: conventional vs. lipid-formulations. *Clin Infect Dis 38:* 377–383.
12. Berman JD, Badaro R, Thakur CP, Wasunna KM, Behbehani K, Davidson R, Kuzoe F, Pang L, Weerasuriya K, Bryceson ADM, 1998. Efficacy and safety of liposomal amphotericin B (AmBisome) for visceral leishmaniasis in endemic developing countries. *Bull World Health Organ 76:* 25–32.

13. Dietze R, Fagundes S, Brito E, Milan EP, Feitosa TF, Suassuna FAB, Fonschiffrey G, Ksionski G, Dember J, 1995. Treatment of kala-azar in Brazil with Amphocil (amphotericin B cholesterol dispersion) for 5 days. *Trans R Soc Trop Med Hyg 89:* 309–311.
14. Gaeta GB, Maisto A, Di Caprio D, Scalone A, Pasquale G, Felaco FM, Galante D, Gradoni L, 2000. Efficacy of amphotericin B colloidal dispersion in the treatment of Mediterranean visceral leishmaniasis in immunocompetent adult patients. *Scand J Infect Dis 32:* 675–677.
15. Sundar S, Jha TK, Thakur CP, Mishra M, Singh VP, Buffels R, 2002. Low-dose liposomal amphotericin B in refractory Indian visceral leishmaniasis: a multicenter study. *Am J Trop Med Hyg 66:* 143–146.
16. Sundar S, Agrawal G, Rai M, Makharia MK, Murray HW, 2001. Treatment of Indian visceral leishmaniasis with single or daily infusions of low dose liposomal amphotericin B: randomized trial. *BMJ 323:* 419–422.
17. Sundar S, Jha TK, Thakur CP, Mishra M, Singh VP, Buffels R, 2003. Single-dose liposomal amphotericin B in the treatment of visceral leishmaniasis in India: a multicenter study. *Clin Infect Dis 37:* 800–804.
18. Syriopoulou V, Daikos GL, Theodoridou M, Pavlopoulou I, Manolaki AG, Sereti E, Karamboula A, Papathanasiou D, Krikos X, Saroglou G, 2003. Two doses of a lipid formulation of amphotericin B for the treatment of Mediterranean visceral leishmaniasis. *Clin Infect Dis 36:* 560–566.
19. Jha TK, Olliaro P, Thakur CP, Kanyok TP, Singhania BL, Singh IJ, Singh NKP, Akhoury S, Jha S, 1998. Randomised controlled trial of aminosidine (paromomycin) v. sodium stibogluconate for treating visceral leishmaniasis in North Bihar, India. *BMJ 61:* 1200–1205.
20. Sundar S, Jha TK, Thakur CP, Engel J, Sindermann H, Fischer C, Junge K, Bryceson A, Berman J, 2002. Oral miltefosine for Indian visceral leishmaniasis. *N Engl J Med 347:* 1739–1746.
21. Pagliano P, Rossi M, Rescigno C, Altieri S, Coppola MG, Gramiccia M, Scalone A, Gradoni L, Faella F, 2003. Mediterranean visceral leishmaniasis in HIV-negative adults: a retrospective analysis of 64 consecutive cases (1995-2001). *J Antimicrob Chemother 52:* 264–268.
22. Pintado V, Martin-Rabadan P, Rivera ML, Morneo S, Bouza E, 2001. Visceral leishmaniasis in human immunodeficiency virus (HIV)-infected and non-HIV-infected patients. A comparative study. *Medicine (Baltimore) 80:* 54–73.
23. Laguna F, 2003. Treatment of leishmaniasis in HIV-positive patients. *Ann Trop Med Parasitol 97 (suppl 1):* 135–142.
24. Ritmeijer K, Veeken H, Melaku Y, Leal G, Amsalu R, Seaman J, Davidson RN, 2001. Ethiopian visceral leishmaniasis: generic and proprietary sodium stibogluconate are equivalent; HIV co-infected patients have a poor outcome. *Trans R Soc Trop Med Hyg 95:* 668–672.
25. Badaro R, Nascimento C, Carvalho JS, Badaro F, Russo D, Ho JL, Reed SG, Jones TC, 1994. Recombinant human granulocyte-macrophage colony-stimulating factor reverses neutropenia and reduces secondary infections in visceral leishmaniasis. *J Infect Dis 170:* 413–418.
26. Croft SL, Coombs GH, 2003. Leishmaniasis - current chemotherapy and recent advances in the search for novel drugs. *Trends Parasitol 19:* 502–508.
27. Demicheli C, Ochoa R, da Silva JB, Falcao CA, Rossi-Bergmann B, de Melo AL, Sinisterra RD, Frezard F, 2004. Oral delivery of meglumine antimonoate-beta-cyclodextrin complex for treatment of leishmaniasis. *Antimicrob Agents Chemother 48:* 100–103.