REVIEW ARTICLE

Infections in AIDS

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Introduction

Reports of an unusual syndrome in which previously healthy young males were presenting with diseases previously important only in immunocompromised individuals appeared in 1981 [1]. The diseases were cytomegalovirus pneumonia, *Pneumocystis carinii* pneumonia and Kaposi's sarcoma and the condition became known as the acquired immunodeficiency syndrome or AIDS. Since then the numbers have increased inexorably with an estimated 1 736 958 cumulative AIDS cases world-wide in 1997 [2]. The same survey also estimated that in Nov. 1997 there were 30.6 million people living with HIV/AIDS in the world [2]. Most of these were in subSaharan Africa (20.8 million: 68%), South and South-East Asia (6 million: 19.6%) and Latin America (1.3 million: 4.2%), with smaller numbers in North America (860 000: 2.8%), Western Europe (530 000: 1.7%) and East Asia and the Pacific (440 000: 1.4%).

Although HIV itself can produce disease manifestations, its major effect is the diminution of immunity leading to the appearance of opportunist infections and tumours. Indeed, the AIDS epidemic has resulted in the recognition of a number of new pathogens. Table 1 illustrates the range of pathogens that have been recognised as causing opportunist infections in AIDS.

Different opportunist infections or conditions present at different times in the evolution of the infection with HIV. Thus, invasive pneumococcal and non-typhoidal salmonella infections and oral candidosis present early in HIV-infected patients, especially in Africa. The appearance of chronic muco-cutaneous herpes simplex infection, oesophageal candidosis, chronic cryptosporidial diarrhoea, wasting syndrome and Kaposi's sarcoma were the commonest conditions defining entry of HIV-infected patients into WHO stage 4 (AIDS) in a Ugandan study of adults [3]. In contrast, in infants with

AIDS, *Pneumocystis carinii* pneumonia is a common cause of morbidity and mortality [4], with up to 30% of children under 1 year having evidence of infection at post mortem [5].

The introduction of highly active antiretroviral therapy (HAART) has radically altered AIDS incidence in developed countries, and thus the impact of opportunist infections [6, 7]. In the initial flush of enthusiasm over HAART some commentators even suggested that it might not just cure AIDS but also, by completely preventing HIV replication, eventually eliminate the virus as infected cells died. As might be expected these hopes have not been realised. Firstly, some of the HAART regimens have not proved as effective in children as in adults, e.g., viral loads decreased to <400 copies/ml in as few as 20% of those treated and rebounded in as many as 44% of those successfully treated [8–10]. However, this problem has been resolved by employing different regimens (e.g., a combination of efavirenz, nelfinavir and nucleoside reverse transcriptase inhibitors) [11]. However, a larger problem is the emergence of drug-resistant mutants. During acute infection it is estimated that *c.* 10⁹ HIV particles are produced per day and *c.* 10⁶ of these are mutants [12, 13]. Thus it is clear that application of selective pressure such as antiviral chemotherapy will drive the development of resistant mutants as happened, for example, with zidovudine monotherapy [14]. Ominously, in a recent study 16% of newly diagnosed cases of HIV-1 infection were with antiretroviral-resistant genotypes [15]. Combining antiretroviral agents was expected to decrease the chance of resistance developing. The administration of HAART is accompanied by a great decrease in plasma HIV load and has been shown to obviate the need for chemoprophylaxis of *P. carinii* pneumonia [16]. However, it is now clear that even with HAART, which results in undetectable plasma HIV loads, HIV transcription still persists in peripheral blood mononuclear cells. In addition, HIV may also persist in cells in semen of patients on HAART who have undetectable plasma HIV loads [18].

Table 1. Some opportunist pathogens in AIDS

Viruses	Bacteria	Fungi	Protozoa	Other
Human herpesviruses 1–5 & 8	Anaerobes	<i>Aspergillus</i> spp.	<i>Acanthamoeba</i> spp.	<i>Sarcoptes scabiei</i>
Human papillomavirus	<i>Bartonella henselae</i>	<i>Candida albicans</i>	<i>Cryptosporidium parvum</i>	<i>Strongyloides stercoralis</i>
JC virus	<i>B. quintana</i>	<i>Candida</i> spp.	<i>Entamoeba histolytica</i>	
Molluscum contagiosum	<i>Bordetella bronchiseptica</i>	<i>Coccidioides immitis</i>	<i>Giardia lamblia</i>	
	<i>Campylobacter</i> spp.	<i>Cryptococcus neoformans</i>	<i>Isospora belli</i>	
	<i>Chlamydia trachomatis</i>	<i>Blastomyces dermatidis</i>	<i>Microsporidia</i>	
	<i>Clostridium difficile</i>	Dermatophytes	<i>Toxoplasma gondii</i>	
	<i>Haemophilus influenzae</i>	<i>Cryptococcus neoformans</i>		
	<i>Legionella pneumophila</i>	<i>Histoplasma capsulatum</i>		
	<i>Listeria monocytogenes</i>	<i>Malassazia furfur</i> (?)		
	<i>Moraxella catarrhalis</i>	<i>Mucor pusillus</i>		
	<i>Mycobacterium tuberculosis</i>	<i>Penicillium marneffei</i>		
	<i>M. avium intracellulare</i>	<i>Pneumocystis carinii</i>		
	<i>M. fortuitum</i>	<i>Sporothrix schenckii</i>		
	<i>M. kansasii</i>			
	<i>M. marinum</i>			
	<i>Neisseria gonorrhoeae</i>			
	<i>Nocardia asteroides</i>			
	<i>Rhodococcus equi</i>			
	Salmonellae (non-typhi)			
	<i>Shigella</i> spp.			
	<i>Staphylococcus aureus</i>			
	<i>Streptococcus pneumoniae</i>			
	<i>Treponema pallidum</i>			

The presence of HIV replication in privileged sites such as seminal cells and CSF and even peripheral blood mononuclear cells during HAART allows the possibility of development of resistant mutants. Although none was detected in the systematic studies of HIV persistence in HAART [17–19], mutants resistant to reverse transcriptase inhibitors (nucleoside and non-nucleoside) and protease inhibitors have been detected [20, 21]. This problem can be ameliorated to a certain extent by using resistance genotyping to guide initiation and changes in combination therapies [22]. Nevertheless, even with ostensibly sensitive HIV, HIV load is not reduced to undetectable levels in up to 10% of patients and 20% experience viral load rebound [23]. Interestingly, such virological failures were not necessarily associated with clinical progression of AIDS, but such occurrences are bound to increase the risk of resistance developing.

Although in developed countries the incidence of opportunist infections has greatly diminished following the introduction of HAART, it is clear that this may not always be the case. Furthermore, in tropical countries where the greatest burden of HIV disease occurs, HAART is unfortunately not affordable and opportunist infections abound. Therefore, it seemed appropriate to have infections in AIDS as the theme of the sixth symposium on microbial disease.

MICROSPORIDIOSIS

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Microsporidia are regarded as almost ubiquitous in the wild, particularly amongst invertebrates [24] and fish

[25], but some have also been described in birds and mammals [25]. Some were known to cause significant economic loss to commercial producers of honey, silk and fish if their animal stocks were infected. Against this widespread occurrence in nature, only a handful of human infections had been documented before 1985 and so this group of parasites had been largely ignored by the medical world. However, this paradox has now evaporated with the advent of AIDS and the recognition of HIV infection, as significant numbers of human infections involving these parasites have become recognised. Many of these infections involve species that were previously unknown. Interestingly, as a recent study has shown a high seroprevalence to *Encephalitozoon* spp. in the immunocompetent population [26], subclinical infection or infection involving mild, transient symptoms may be common in man.

Microsporidiosis comprises a group of infectious diseases caused by microsporidia that occur predominantly, but not exclusively, in human patients with AIDS. So, what are microsporidia? Microsporidia are small, unicellular, obligately intracellular parasites that are transmitted as highly resistant spores. The spores contain the infective sporoplasm and a coiled tube (the polar tube) with associated extrusion apparatus, the presence of which is pathognomonic of microsporidial infection. On entering a suitable host, the polar tube is everted and penetrates a susceptible host cell. The sporoplasm migrates through the everted polar tube and is injected into the host cell cytoplasm. Replication is a two-stage process, initially involving repeated proliferation (merogony) before commitment to spore production (sporogony). About 1000 species are known currently, but this is thought to be a gross underestimate of the total number of species within the phylum Microspora [24]. Indeed, new species are still

being described from many hosts at a significant rate, year on year.

Chronic diarrhoea, malabsorption and wasting are common presentations in AIDS patients. In 1985, *Enterocytozoon bieneusi* (Fig. 1) was first described [27] and this microsporidian has become recognised as the commonest microsporidian species reported to occur in man. Most infections either occur or become clinically significant when the CD4 lymphocyte count falls below $100 \times 10^6/L$ [28]. A second enteric species, *Encephalitozoon (Septata) intestinalis*, was described in 1993 [29] and dual infections involving both these species have been reported [30]. *Ent. bieneusi* is normally restricted to the small intestinal enterocytes, whereas *E. intestinalis* spreads into the lamina propria [29]. Both these species have been found to spread from the intestine along the epithelium to the gall bladder and the pancreatic and bile ducts [29, 31]. Although rarer, spread to the respiratory system has been documented with both species [31, 32]. Most studies [33, 34], but not all [35], have shown an association between enteric infection and symptoms of diarrhoea and weight loss.

Several microsporidian infections of the eyes have been reported (Table 2). In AIDS patients, ocular involvement is often the presenting sign of microsporidiosis. Infection of corneal and conjunctival epithelia commonly involves *E. hellem* (Fig. 2) [36], but *E. intestinalis*, *E. cuniculi* and *Trachipleistophora hominis* have also been reported. These infections cause a bilateral punctate keratopathy (keratoconjunctivitis) [37]. *E. hellem* has also been reported from the lungs [38], which may be the source of some ocular

Fig. 1. Enterocyte from a small intestinal biopsy from an AIDS patient infected by *Enterocytozoon bieneusi* showing spores (densely stained) and a merogonic stage (M). Note that this parasite is in intimate contact with the host cell cytoplasm. Magnification 16 000 $\times$.

Table 2. Species and sites of microsporidian infections in man

Organism	Main sites of infection
<i>Enterocytozoon bieneusi</i>	Small intestinal epithelium, bile duct epithelium and, rarely, nasal polyps and bronchial epithelium
<i>Encephalitozoon cuniculi</i>	Liver, peritoneum, kidney, intestine, eyes
<i>Encephalitozoon hellem</i>	Corneal and conjunctival epithelia, nasal polyps, kidney, tracheobronchial tree
<i>Encephalitozoon intestinalis</i> (syn. <i>Septata intestinalis</i>)	Epithelia of the gut from small intestine to colon, macrophages in the lamina propria, kidney, eyes and gall bladder
<i>Trachipleistophora hominis</i>	Skeletal muscle, heart muscle, corneal epithelium, kidney, nasopharynx
<i>Trachipleistophora anthrophophthera</i>	Brain, kidney, heart, pancreas, thyroid, parathyroid, liver, spleen, bone marrow
<i>Pleistophora</i> spp.	Skeletal muscle
<i>Brachiola vesicularum</i>	Skeletal muscle
<i>Brachiola connori</i> (synonym for <i>N. connori</i>)	Disseminated, defective lymphoid system, smooth and cardiac muscle, kidney, liver, lungs, adrenal cortex
<i>Vittaforma corneae</i> (syn. <i>N. corneum</i>)	Corneal stroma of the eye
<i>Nosema oculorum</i>	Corneal stroma of the eye
' <i>Microsporidium</i> ' <i>ceylonensis</i>	Corneal stroma of the eye
' <i>Microsporidium</i> ' <i>africanum</i>	Corneal stroma of the eye

Fig. 2. Nasal epithelium from an AIDS patient showing various stages in the development of *Encephalitozoon hellem*. Note that development is undertaken in a parasitophorous vacuole. Magnification 8000 $\times$.

infections by reverse passage from this respiratory site of infection [39]. Of the reported stromal infections, most have been unrelated to HIV infection [25]. However, *Vittaforma corneae* [40] was originally isolated from an immunocompetent patient (as *Nosema corneum* [41]) and infection was established in athymic mice [42]. On this basis, it was predicted that *V. corneae* could become established in immunosuppressed human patients [42] and this species has recently been reported from an AIDS patient [43].

Another presentation can be myositis. *Pleistophora*-like species [44, 45], *T. hominis* (Fig. 3) [46, 47], *Brachiola vesicularum* and *B. connori* [48] infections produce symptoms of severe muscle pain and weakness. Such microsporidian infection is associated with myocyte destruction. *T. hominis* and *B. vesicularum* have only recently been described. Only in the case of *B. vesicularum* was infection restricted to skeletal muscle. A second species of *Trachipleistophora*, *T. anthropopthera*, is the latest microsporidian to be described in man. The parasite was found in the brain of two AIDS patients [49], a site of infection that has been documented infrequently in previous human microsporidian infections. Currently, about 13 microsporidian species have been identified in man (Table 2), most, but not all, associated with HIV infection. These parasites are now known to infect individual organs such as gut, eyes, brain, skeletal muscle, heart, tracheobronchial tree, liver, kidneys and genitourinary tract, producing a variety of clinical symptoms, such as diarrhoea, cholangitis, bronchitis, sinusitis, keratoconjunctivitis, hepatitis, myositis, encephalitis, nephritis and urethritis [31, 50]. Other sites of infection and associated symptoms are likely to be found with human microsporidian infection. Disseminated infection is now widely recognised [29, 31, 51] and without treatment such infection can be significantly debilitating and may have a fatal outcome. The possibility of disseminated infection should be considered particularly if species of *Encephalitozoon* are diagnosed [38]. The mechanism of dissemination is not understood, but is thought to involve macrophages [51].

As might be expected from the nature and course of HIV infection, antibodies appear to be significantly less protective than the cell-mediated immune response in

Fig. 3. Pectoral muscle from an AIDS patient showing sporophorous vesicles (SPOVs) containing spores of *Trachipleistophora hominis*. Magnification 6500 $\times$.

controlling microsporidian infections [38]. Experimental infections in athymic mice have confirmed this view [52].

One of the most fascinating aspects of microsporidian infection in man is the determination of possible sources of such infections. Contaminated food and water are likely vehicles of transmission to man by the faecal–oral or urine–oral route, but sources of human microsporidian infection remain largely obscure. Acquisition through the respiratory epithelium has also been suggested [53], as has introduction through eye abrasions [39]. Some may be natural human parasites, but animal sources are likely for the rest. Some human microsporidian infections may result from rare accidental exposure to microsporidian spores (such as in infected fish muscle) and only become established if the human host is immunocompromised. Species of *Pleistophora* are common muscle infections in fish [25] and crustacea (shrimps and crabs) [24] and such food sources may account for the small number of human microsporidian muscle infections involving *Pleistophora*-like species identified to date [44–46]. Some microsporidia, such as *E. cuniculi*, infect many mammals, particularly rodents, rabbits and dogs [25]. *E. cuniculi* infection in one AIDS patient was confirmed to be a dog strain by molecular methods, which indicates that some pets may be a potential source of human infection [54]. Other pets, such as caged birds, may be sources of infection, as *E. hellem* has recently been identified in the intestinal epithelium of budgerigars [55] and in the intestine and also the kidneys of parrots [56]. Interestingly, the parrots were co-infected with a virus that induced immunosuppression [56]. As the *E. hellem* infection in these birds involved the intestine, the potential for human contact with droppings containing spores is self-evident. As such droppings are generally dry, or dry quickly after being voided, inhalation of dust containing spores into the respiratory tract is a distinct possibility. Pets can and do provide a source of comfort to their human owners but, perhaps, caring for certain pet animals (such as dogs [54], rabbits [57] and caged birds [55, 56]) should be discouraged if the individual is HIV-infected. However, with a high seroprevalence for some microsporidian species, particularly *Encephalitozoon* spp. [26] and the possibility of reactivation of latent infection [58], then perhaps this advice is only pertinent in preventing exposure to potential new sources of infection involving animal species. Faeces from domestic farm livestock, including donkeys, pigs, cows and goats, have recently been shown to contain spores of *E. intestinalis* in a study in a rural village in Mexico [59]. The commonest human microsporidian parasite, *Ent. bienersi*, has been identified in both pigs [60] and macaque monkeys with simian AIDS [61], but neither of these sources could explain the high prevalence in AIDS patients. The demonstration of four genetically diverse variants of *Ent. bienersi* [62] suggests that much remains to be learned about the

epidemiology and host range of this parasite. The possibility that these animal infections have been acquired from their human handlers also needs to be examined. Another possibility is transmission of microsporidia from arthropod sources, particularly blood-sucking or biting insects. Whether microsporidia from such poikilothermic sources can infect warm-blooded mammals, such as man, has not been determined. However, as a microsporidian parasite of mosquitoes has been successfully transmitted to mice, this may be a possibility, particularly in immunosuppressed individuals [63]. Work in this area is still at an early stage and is summarised in Table 3.

Diagnosis of microsporidial infections in tissue biopsies can be difficult because of their small size, intracellular nature and poor staining properties with normal histological stains. Transmission electron microscopy (TEM) can be useful here, but availability may be a problem [34]. Spores of *Ent. bienewsi* are the smallest reported to infect man, being only *c.* $1.8 \times 1 \mu\text{m}$. Such spores can be detected in faeces or body fluids by means of calcofluor [64] or trichrome stains [65]. Some workers have advocated using calcofluor as a screening stain, followed by trichrome as a confirmatory stain [66]. Where specialised laboratory techniques are unavailable, this is acceptable, but a significant disadvantage of these light microscopy methods is that the species involved cannot be determined. Species determination is important as some infections can be treated successfully, whereas others involving different species may not (see below). Availability of commercial, species-specific fluorescein-labelled antibodies would be a significant advantage here and some are becoming available [67]. Electron microscopy or molecular methods should be used as confirmatory tests, if available. Most workers utilising molecular diagnostic methods in the field of microsporidial detection have amplified parts of the small subunit ribosomal RNA gene [68]. Continued use of molecular techniques, such as PCR, will significantly improve our knowledge of the pathogenesis, epidemiology and phylogeny of these opportunist parasites.

Some microsporidial infections (particularly those involving *Encephalitozoon* spp.) are treatable with the

current treatment of choice, albendazole [69–71]. Albendazole inhibits polymerisation of the intranuclear spindle microtubules during nuclear division, thus preventing chromosome separation. Parasite division is thus inhibited, but this does not appear to have a parasitocidal action [72]. However, in human patients, infection with *Encephalitozoon* spp. can be treated successfully [73] but *Ent. bienewsi*, in particular, is often refractory to treatment (although there can be symptomatic improvement) and more effective treatment is required. Several new compounds show promise [74]. Fumagillin has been used topically to treat eye infections caused by *E. hellem* [75] but was thought to be too toxic for treatment of other human microsporidial infections. However, in a recent trial fumagillin successfully eradicated infection due to *Ent. bienewsi* but caused thrombocytopenia in the treated patients [76]. A semisynthetic analogue of fumagillin, TNP-470, which is less toxic, has also been shown to be effective against *E. cuniculi* in infected cell culture and an athymic nude mouse model [77]. A controversial drug from the past, thalidomide, also appears to have anti-microsporidial activity [78]. Faecal tumour necrosis factor- α (TNF- α) is elevated in AIDS patients with enteric microsporidial infection. In one trial, thalidomide, a potent anti-TNF- α agent, was shown to effect a complete or partial clinical response in over half the AIDS patients with chronic diarrhoea due to *Ent. bienewsi* who were treated.

With the recent improvements in antiretroviral treatment of AIDS patients [79] in the developed world, degradation of the cellular immune system has been halted and reconstitution has had a significant downward effect on opportunist parasite prevalence rates [80]. A study from the UK, based on data from 1992 to 1995, showed a prevalence rate of the enteric parasite *Ent. bienewsi* in AIDS patients of *c.* 15% [34], but other studies have shown higher rates. This UK-based study ended in Dec. 1995 and only a single further case has been diagnosed to date from the same source (unpublished observations). This decrease appears to correlate with the improved antiretroviral treatment now administered to AIDS patients [79]. If these improved treatment regimens remain effective, occurrence of such opportunist infections will remain low. Such a reduction in opportunist infections will, of course, affect the demand for newer (PCR-based) diagnostic tests and thus the occasional suspected infection will be diagnosed only by conventional microscopic methods. However, in the developing world the situation is very different and is a cause for concern [79].

Much remains to be learned about this group of emerging pathogens in relation to human infection and disease. Even basic information such as the incubation period of the various species of parasites in man is not known. Most of the current knowledge about human microsporidial infections has come from AIDS pa-

Table 3. Human microsporidian species and identified animal hosts

Organism	Animal host
<i>Enterocytozoon bienewsi</i>	Domestic pigs, Macaque monkeys with AIDS
<i>Encephalitozoon cuniculi</i>	Wide host range among mammals, including rodents, rabbits and dogs
<i>Encephalitozoon hellem</i>	Budgerigars, parrots
<i>Encephalitozoon intestinalis</i> (syn. <i>Septata intestinalis</i>)	Dog, donkey, pig, cow and goat
<i>Pleistophora</i> spp.	Many fish and crustacea

tients. Patients who become immunosuppressed from other causes, such as malignancy or its treatment or organ transplantation, are also becoming recognised as potential hosts for microsporidia [81–83]. Because diagnostic methods have been developed and workers are now looking for them, microsporidia are also being recognised increasingly in the immunocompetent population, particularly in relation to traveller's diarrhoea, where both *Ent. bienersi* [84, 85] and *E. intestinalis* [86] have been identified. Such cases suggest that enteric microsporidial infections, at least, may be much commoner causes of sporadic diarrhoea than has been suspected previously. The field of microsporidial research, particularly in relation to human infection, is a rapidly expanding field of interest and we can expect many more interesting developments in the near future.

INVASIVE PNEUMOCOCCAL DISEASE IN HIV-INFECTED ADULTS

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Introduction

Streptococcus pneumoniae is an important and dangerous infectious complication of HIV infection, and together with non-typhoidal salmonellae the most important complicating bacterial pathogen. *S. pneumoniae*-related illness was absent from the earliest descriptions of AIDS in North America. However, with the ability to detect HIV infection before AIDS and the introduction of anti-retroviral agents and prophylactic therapies, the spectrum of HIV/AIDS-related illness has changed. The incidence of *Pneumocystis carinii* pneumonia (PCP) has fallen, and bacteria, most importantly *S. pneumoniae*, have played an increasingly important role [87–90]. Bacterial pneumonia is now amongst the leading causes of HIV-related admission to hospital in the USA [91–97].

The association of HIV and *S. pneumoniae* in the developing world is now similarly well recognised [98–102]. Respiratory disease has been, and is, highly prevalent in HIV-infected adults. *S. pneumoniae* and *Mycobacterium tuberculosis* are the leading causes of respiratory illness. Moreover, it is in the developing world where treatment and control of pneumococcal infection are perhaps of greatest importance and present the greatest challenge. The number of HIV-infected adults is large and growing, inadequate secondary health care limits access to appropriate therapy and antimicrobial resistance will continue to develop and spread, the impact of which is worryingly uncertain.

S. pneumoniae is implicated in various clinical syndromes. It is the most frequent bacterial cause of

otitis media, sinusitis and bronchitis [103, 104]. However, it is the invasive syndromes of necrotising pneumonia, bacteraemia and meningitis to which HIV-infected individuals are particularly susceptible, and it is these syndromes which are responsible for the preponderance of *S. pneumoniae*-related mortality. The presentation and management of these serious invasive infections form the focus of this review.

Epidemiology of *S. pneumoniae* and HIV infection

Rates of bacterial pneumonia in HIV-infected adults are increased 5–10-fold compared with age-matched controls [90, 94, 105, 106]. *S. pneumoniae* is invariably the commonest causative agent [94, 96, 107–109]. More importantly, blood stream infection complicates 60–80% [90, 92, 96, 98, 99, 110, 111] of such cases (compared with 10–20% in HIV-uninfected patients [112, 113]). Consequently, bacteraemic pneumococcal disease is between 20 and 100 times more common with HIV/AIDS. In the developing world and amongst intravenous drug users (IVDU), rates of disease may be even higher than those reported amongst homosexual groups in North America [90, 94, 96, 97, 99, 100, 102, 105, 106, 109–11, 115–18] (Table 4). In these groups the environmental and social problems that exist and predispose to increased rates of disease in the HIV-uninfected population are simply compounded by infection with HIV.

Rates of disease increase with advancing immunosuppression. Individuals with AIDS have a three-to-five times greater risk than individuals with asymptomatic HIV infection [95, 111, 119]. Whether reversal of immunosuppression with HAART will decrease the risk of infection is as yet unknown. The recovery of some cellular immune function with this therapy may not translate into improved B-cell function.

Given appropriate access to health care, case fatality rates are similar in HIV-infected and uninfected individuals. Indeed, in North America and Europe the outcome with HIV is better than in age-matched uninfected controls (Table 4). This is probably a consequence of the serious nature of other complicating illnesses in the HIV-uninfected group, i.e., transplant recipients, diabetes mellitus, renal failure, neoplasia, etc. The situation in subSaharan Africa is different. Presentation for treatment tends to be late and case-fatality is high irrespective of HIV status [100, 107].

Recurrence of *S. pneumoniae* infection is frequent. Whilst this phenomenon is not unique to HIV, it occurs at a strikingly high rate. Annual recurrence rates following an episode of invasive infection are between 10 and 30%, and are usually re-infections with different serotypes [99, 110]. The high rate may reflect a subpopulation of HIV-infected individuals who are

Table 4. The 17 principal reports in the literature describing the relationship between HIV and rates of invasive pneumococcal disease (In), pneumococcal pneumonia (Pn) and all bacterial pneumonia (BP) syndromes

Ref. No.	Site	Type of study	Date	Characteristics/ HIV status	Syndrome	Rate	n	Mortality	Recurrence
90	New York	Prospective	1985–86	+ non-AIDS IVDU	Pn	34.6	5	0	...
				– IVDU		3.5	1	0	...
99	Nairobi	Prospective	1989–92	+ CSW	In	23.8	42	0	19
				– CSW		0	0	–	
105	Rome	Prospective	1991–94	+ IVDU	Pn	18.6	30	–	10
				+ IVDU	BP	90.5	149	–	21
				– IVDU	Pn	1.2	5	–	0
				– IVDU	BP	14.2	61	–	2
110	San Francisco	Retrospective community	1983–87	+ Homosexual	In	9.4	75	8	13
				– Age 20–55		0.12	219	18	7
111	New Jersey	Retrospective Community	1986	AIDS-mixed	In	10.7	17	6*	10
				Pre-AIDS-mixed		5.3	–	–	...
				– Age 20–55		0.03	nr	11	...
114	Madrid	Retrospective hospital	1988–90	+ mixed	Pn		21	5	...
				– hospital control			54	19	...
115	New York	Retrospective hospital	1989–90	+ mixed	In		26	31†	...
				– hospital control	Pn		24	25	...
116	Denver	Retrospective hospital	1985–94	+ mixed	In	na	40	8	13
				– hospital control		na	74	5	...
117	New Haven	Retrospective hospital	1992–93	+ mixed	In		25	13	...
				– hospital control			81	12	...
102	Johannesburg	Retrospective hospital	1996	+ heterosexual	In		58	12	...
				– hospital control			71	21	...
96	New York	Retrospective hospital	1986	+ AIDS	Pn	17.9			
				– CDC estimate		2.6			...
100	Addis Ababa	Prospective	1987–89	+ mixed	BP		9	11	...
				– hospital control			101	11	...
118	Denver	Prospective	1991	+ mixed	In	9.4			...
				+ mixed	Pn	20			...
				+ mixed	BP	106			...
106	Amsterdam	Prospective	1991	+ IVDU	BP	290			...
				– IVDU		90			...
97	New York	Prospective	1988–91	+ mixed	BP	48	53	26	...
				– at risk‡		6	1	0	...
94	N. America	Prospective	1988–91	+ mixed	BP	55	181	31	6
				– at risk‡		9	5	20	...
109	Rome	Retrospective case control	1996	+ IVDU	BP		28	23	...
							5		

Recurrence, percentage recurrence; Mortality, percentage case-fatality; n, number of cases; CSW, commercial sex worker; IVDU, intravenous drug user. Rates are expressed per 1000 person years. Mixed population infers all methods of transmission represented.

*All HIV + deaths.

†57% with AIDS died compared with 5% in non-AIDS.

‡Partners of HIV-infected individuals.

unable to mount an effective immune response to polysaccharide antigens and, therefore, have repeated infections. Alternatively, polysaccharide antigens may have a negative impact on B-cell populations by stimulating apoptosis and increasing the susceptibility to further infection. Irrespective of mechanism, the high risk of further disease makes individuals who have had a first episode of *S. pneumoniae* infection a prime target for preventive therapy.

Clinical presentation

S. pneumoniae-related disease is similar in presentation to that in HIV-uninfected adults. Symptoms appear acutely, chest signs are usually present and there is often production of purulent mucoid sputum. These features help to set it apart from PCP, which is a more subacute process often lacking focal chest signs. However, differentiation from tuberculosis may be more difficult, as this may follow a rather atypical and acute presentation, particularly in advanced HIV. In such situations microbiological assessment is essential.

The radiographic appearance of *S. pneumoniae* infection also follows a conventional pattern. A review of 200 cases of bacteraemic pneumococcal pneumonia in HIV-infected and uninfected patients presenting to the Kenyatta hospital in Kenya revealed no significant difference in radiographic appearance between the two groups (unpublished data). The findings were typically those of lobar pneumonia. However, these radiographic findings are also seen in tuberculosis, reinforcing the need to consider carefully the possibility of this infection presenting atypically as lobar pneumonia. Indeed, in the tropics it is not uncommon to find the two infections together.

What is different about *S. pneumoniae* infection in HIV is the increased proportion of non-pulmonary infections leading to invasive disease. Maxillary sinusitis, otitis media and primary occult bacteraemia account for as much as 40% of the invasive episodes [99,110,120]. Moreover, presentations which have become rare in the antibiotic era, i.e., endocarditis, brain abscess, mediastinitis have been reported in HIV-infected individuals [121–123].

Management

Treatment

Response to penicillin-based therapy is usually good, although early treatment is critical. Prompt management of infection in a cohort of HIV-infected commercial sex workers in Nairobi, Kenya where case-fatality rate was running at 15% reduced mortality to zero [99]. This suggests that a high index of suspicion and aggressive early therapy with penicillin are the keys to good outcome and are achievable even

under resource-limited settings. Nevertheless, deaths will still occur. These will usually be in the first 24 h and are a consequence of the established features of sepsis syndrome and the irreversible inflammatory cascade set in motion by the bacterium and its products. Early reports that this was not a feature of pneumococcal sepsis in HIV-infected adults are probably not true, and certainly not the author's experience.

Penicillin resistance is becoming increasingly widespread and is a particular problem in HIV infection. Rates of carriage of antibiotic-resistant *S. pneumoniae* are higher, as are rates of infection with penicillin-resistant bacteria in HIV-infected individuals [102, 108, 114, 117, 124–126]. Despite this, there is little evidence to suggest that outcome is worse in those infected with penicillin-resistant strains. Most resistance is of the intermediate type giving reduced susceptibility and the tissue concentrations achievable with parenteral and oral penicillin antibiotics are likely to achieve bacterial killing. However, antibiotic resistance has tended to be studied in the developed world where bacterial sensitivity testing is widely available and there is ease of access to alternative agents for treatment. The scale of the problem in the developing world is unclear. What little information there is suggests that it is growing, but still predominantly one of reduced susceptibility rather than frank resistance. The loss of penicillins as effective agents against *S. pneumoniae* would be devastating for the management of these infections in resource-poor countries.

Prophylaxis

Prevention of *S. pneumoniae* infection is attractive for several reasons. Rates of disease are high, there is significant associated morbidity and mortality with infection and any inflammatory process may have a negative impact on immune control of HIV. Several methods have been proposed. Vaccination-based strategies have been predicated on the principle that capsule-specific opsonising immunoglobulins are critical to defence against *S. pneumoniae* and that raising levels will provide protection.

Passive vaccination with pooled immunoglobulin. This has been shown to work in symptomatic HIV-infected children but has not been studied in adults [127]. However, expense and the difficulties of administration preclude its use anywhere other than in specialist centres. Moreover, it is a hopelessly impractical approach for the developing world where most cases of paediatric and adult HIV exist.

Active vaccination with polyvalent pneumococcal polysaccharide vaccines. The 23-valent pneumococcal vaccine has been available since the early 1980s. *S.*

pneumoniae of vaccine-preventable capsular serogroups account for 90% of invasive isolates in the HIV-infected population irrespective of geography, so the vaccine developed for North America should also be applicable to the developing world [99, 116, 117, 126, 128]. It has been recommended as a standard of care for HIV-infected adults since 1991 in the UK and since 1989 in the USA. Unfortunately, these recommendations are not based on evidence of vaccine efficacy. Immunological studies have shown that HIV-infected adults can mount a response to polysaccharides contained in the vaccine, although the response is invariably much inferior to that in HIV-uninfected controls [129–134]. The only prospective, blinded and randomised efficacy trial of pneumococcal vaccine in HIV-positive patients has been completed recently in Uganda. The preliminary results of this study suggest that vaccination is ineffective. Indeed, there is some evidence to suggest that vaccination may have increased the risk of *S. pneumoniae* infections and pneumonic illness, casting serious doubts over the safety and use of the vaccine [120]. No data as yet exist on the role of the newer conjugate pneumococcal vaccines. The little work that has been done suggests that the serological response to these vaccines is also poor, although this does not necessarily equate with poor clinical efficacy [135]. Other proposed vaccines based on bacterial peptides are under development; however, the likely cost of such products, if they prove clinically valuable, will put them out of reach of developing countries.

Chemoprophylaxis. This approach has been in widespread use in other groups at increased risk of pneumococcal infection, particularly in individuals with asplenia and sickle cell disease. Penicillin either taken daily or given as monthly injections has been the preferred means of prophylaxis; however, this has not been widely used in HIV infection and there is little published work to support its use. Evidence from case-control studies of co-trimoxazole (used for PCP prophylaxis) does not show a clear benefit with this agent in protecting against bacterial pneumonia [94, 109, 136–139]. More recently two study teams have reported their preliminary findings from prospective work carried out in Cote d'Ivoire. Both studies were randomised, comparing placebo with daily co-trimoxazole. One was performed in HIV-infected adults on TB therapy and the other in a general HIV-infected adult cohort. Both showed a significant effect in reducing respiratory illness [140, 141]. The complete results of these studies are awaited but suggest that daily co-trimoxazole may be useful in controlling *S. pneumoniae* infections in Africa. However, large numbers of people receiving daily co-trimoxazole will significantly affect antimicrobial resistance patterns in all bacterial populations and there may be unforeseen public health consequences of this strategy.

Conclusion

S. pneumoniae is a ubiquitous human pathogen and will continue to be an important HIV-associated problem. Whether better HIV control in the developed world will reduce susceptibility to infection is unknown; it will certainly increase the number of individuals at risk. The failure of the 23-valent polysaccharide vaccine is a major setback, particularly in the developing world where it offered the most realistic hope of achieving control of *S. pneumoniae* infections. A better understanding of the host–bacteria interaction and newer vaccines may provide a fresh focus for prevention, but there is no immediate promise of success. The enthusiasm to promote mass chemoprophylaxis has to be tempered by the provisional nature of efficacy results, and the potential for adverse effects on public health and bacterial ecology. Consequently management in the foreseeable future will continue to rely on the knowledgeable and alert clinician.

NON-TYPHOIDAL SALMONELLAE IN AIDS

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Salmonella enterica comprises a large number of serovars. *S. enterica* serovars Typhi and Paratyphi A, B and C cause typhoid and paratyphoid fevers, respectively, in which the pathogens penetrate through the intestinal mucosa, produce bacteraemia and lodge in the macrophages of the reticulo-endothelial system. The remaining *c.* 2200 serovars are generally considered zoonotic and are causes of diarrhoeal disease. Nevertheless, even these non-typhoidal salmonellae (NTS) can cause bacteraemia.

Even in well-nourished children in developed countries, up to 24% infected with NTS experience transient asymptomatic bacteraemia [142, 143]. In contrast, studies in African children have shown repeatedly that NTS are the commonest cause of bacteraemia, being responsible for 40–60% of cases [144]. Here, factors predisposing to the development of NTS septicaemia include malaria, severe malarial anaemia, sickle cell disease, malnutrition and schistosomiasis [145, 146–148]. HIV infection does not appear to play a major role in susceptibility to NTS bacteraemia in African children [144, 146]. However, there is a suggestion that relapse of NTS bacteraemia after completion of appropriate antimicrobial chemotherapy is more common in HIV-infected children [147].

In contrast, NTS septicaemia is one of the most frequent manifestations of HIV disease in adults in Africa [98, 149–152] and elsewhere [153]. In a Kenyan study, a total of 138 NTS isolations were made from 1220 episodes of fever in adults admitted to the Kenyatta Hospital, Nairobi [150]. This represented 11.3% of all blood cultures and well over 50% of positive blood cultures. A total of 224 (19.7%) of the 1220 patients with febrile episodes were HIV seropositive, but 95 (68.8%) of the 138 with NTS bacteraemia were HIV infected, demonstrating a strong association ($p < 0.001$) between being HIV seropositive and having NTS bacteraemia [150]. In Malawi NTS were found to be a major cause of bacteraemia and of mortality in adults and showed an overwhelming association with HIV infection [152]. Studies in the USA have estimated that NTS bacteraemia is 100-fold more common in HIV-infected adults than in the general population [153, 154]. There are case reports of focal NTS infections in HIV, including endocarditis [155], intracranial infections [156] and pyomyositis [157]. Pulmonary involvement in HIV-infected patients with NTS bacteraemia is well recognised, and may represent either isolated NTS lung disease or co-infection with a second respiratory pathogen [158]. Despite these isolated reports, most case series find that focal metastatic NTS infections in HIV are rare [157, 159]. Presentation may mimic enteric fever [107], and a lack of diarrhoea or other gastrointestinal symptoms is commonly reported in HIV-positive patients [157, 159].

Relapse of infection is an AIDS-defining event. Between 22% [159] and 44% (Gordon *et al.* unpublished data) of patients have recurrent bacteraemia reported, in a median time of 87 days [159]. Interestingly, in a Spanish study, relapse was least likely to occur in patients receiving zidovudine and the antiviral agent was shown to be bactericidal at therapeutically achievable concentrations [159]. The role of antibiotic therapy in preventing relapses is unclear.

The bacteria

There are well over 2000 serovars of the zoonotic *S. enterica*, but Typhimurium and Enteritidis are those implicated most frequently in invasive disease in AIDS. In Kenya, 75% of the blood isolates from adults with AIDS were Typhimurium with smaller proportions of Enteritidis (9.5%), Newport (8.4%) and Choleraesuis (3.6%) [150] and a similar pattern was found in Malawi [152]. However, in a recent study from Spain, 65% of the NTS blood isolates were Enteritidis and only 27% were Typhimurium [159].

In developed countries one particular phage type or clone of Typhimurium or Enteritidis tends to be responsible for most cases of salmonellosis. For example, Typhimurium definitive phage type (DT)

204C was the predominant cause of salmonellosis in man and livestock in the 1980s in the UK [160]; this has now been superseded by DT104 [161]. Enteritidis PT4 associated with poultry and their eggs is the predominant cause of human infection from these sources [162]. In contrast, in Nairobi among the Typhimurium isolates there were 11 different DTs and 31% were untypable or reacted non-specifically with phages. There was a predominance of DT 56, but this represented only 27% of the isolates [163]. However, PFGE of macrorestricted chromosomal DNA further subdivided the isolates into different genotypes. For example, there were five separate genotypes of DT 56 and four of DT 193 [163]. It does appear that invasive salmonellosis in AIDS patients in Kenya, at least, does not result from dissemination of a single clone of Typhimurium, rather multiple different clones are responsible.

Antimicrobial susceptibility

In developed countries there is great concern over the development of antimicrobial resistance among zoonotic salmonellae. For example, Typhimurium DT 104 has a multidrug resistance phenotype encoded on a chromosomal integron [164] and there has been an increase in fluoroquinolone resistance among some *Salmonella* serovars [165]. Resistance is also a problem in some developing countries. For example, in Nairobi between 48% and 56% of the isolates were resistant to three or more of the antimicrobial agents routinely available in Kenya for therapy [150, 163]. Overall, 48% were resistant to ampicillin, 35% to cefuroxime, 49% to streptomycin, 46% to co-trimoxazole, 26% to chloramphenicol and 66% to tetracycline. Resistance was transferable on *c.* 100-kb plasmids of differing incompatibility and restriction endonuclease digest patterns [150]. Fortunately, resistance to fluoroquinolones (which are not readily available in Kenya) is rare [150, 163], but the current levels and extent of resistance pose considerable therapeutic problems.

Bacterial pathogenicity

Recently, it has become clear that pathogenic bacteria have acquired clusters of virulence genes called pathogenicity islands [166, 167]. Pathogenicity islands (PI), have a different G+C content from the rest of the chromosome (or plasmid in the case of *Yersinia pestis* or *Shigella dysenteriae*), are often flanked by invert repeats and are inserted close to tRNA genes, which suggests that they are of exogenous origin. Such PIs often encode type III secretion systems which are assembled and dismantled according to environmental conditions and inject molecules into eukaryotic host cells modifying their activity [167]. *S. enterica* has at least five PIs and a number of smaller islets [168–172]. Their functions are gradually being unravelled (Table 5);

Table 5. Salmonella pathogenicity islands (SPI)

Characteristics	SPI-1	SPI-2	SPI-3	SPI-4	SPI-5
Chromosomal location	63 min (not close to tRNA gene)	30.7 min (<i>valV</i> tRNA gene)	82 min(<i>selC</i> tRNA gene)	92 min (putative tRNA gene)	20 min (<i>serT</i> tRNA gene)
Size	40 kb (30 ORF)	40 kb (32 ORF)	17 kb (10 ORF)	25 kb (18 ORF)	3.8 kb (6 ORF)
Secretion system	Type III and ABC iron transporter	Type III	?	Type I	Nil
Secreted proteins	SipA, Spt B SopB, AvrA	SpiC	MgtC	?	Sop B
Function	Penetration into enterocytes	Invasion into tissues, inhibition of phagosome fusion (SpiC)	Intramacrophage survival, growth at low [Mg ²⁺]	Intramacrophage survival? induction of apoptosis	Diarrhoeal disease

however, it is clear that there is a degree of redundancy, i.e., some activities are encoded by more than one PI. Some are unstable (e.g., SPI-2) and some encode different genes depending on the serovar (e.g., SPI-3). Whether there are differences between NTS causing invasive disease and those causing just diarrhoeal disease is not known.

Transmission, colonisation and invasion

Although it is assumed that NTS infections are acquired as food-borne zoonoses, this might not be the case in developing countries. An extensive survey of cattle, goat, camel, pig and poultry farms and abattoirs, wild rodents, food samples and water-courses draining farms and abattoirs, has detected only a few colonised animals or contaminated food or water samples (Kariuki *et al.* unpublished data). When serotyped and genotyped, the NTS obtained were unrelated to those isolated from patients. Whether person-to-person transmission is a major route of transmission in developing countries is not clear.

The portal of entry is presumed to be the gastro-intestinal tract either through M cells or directly through enterocytes. However, it is noteworthy that not all individuals with invasive NTS infection, either children or HIV-infected adults, have concurrent diarrhoea or isolation of the bacteria from stool [144, 146, 159, 173]. Thus, in a Spanish study, only 34% of AIDS patients with NTS bacteraemia had diarrhoea and salmonellae were isolated from the stools of only 4% [159]. Pharyngeal colonisation of children by enteric bacteria, at least in developing countries, is not uncommon [174] and more frequent in those immunocompromised by malnutrition [175]. This is an area rich in lymphoid tissue that might also be a portal of entry for NTS.

From the intestine, NTS reach the bloodstream *via* lymphatics. In addition to invasion by penetration of M cells and epithelial cells, it has recently been reported that NTS can also traverse the gut mucosa and disseminate extra-intestinally to the liver and spleen *via* CD18-expressing phagocytes (monocyte-macrophage lineages), inside which the bacteria survive

[176]. This could conceivably facilitate reticulo-endothelial persistence of NTS during antimicrobial chemotherapy, leading to relapsing infection.

Although the impressive array of virulence determinants, which also include plasmid-encoded genes (*spv*) and flagella, expressed by NTS are of major importance, the immune deficit in HIV-infected patients clearly contributes to invasive disease. Interferon- γ and interleukin-12 (IL-12) are essential for monocyte/macrophage control of intracellular infections including NTS, and patients with congenital deficiencies in interferon- γ or IL-12 protein or receptor components are susceptible to NTS bacteraemia [177, 178]. IL-12 expression by peripheral blood mononuclear cells is decreased in HIV infection [179] and IL-10, which down-regulates IL-12 production, is over-expressed in HIV infection [180]. Thus, at least one of the key factors involved in diminishing the severity of NTS infection is suppressed in HIV infection. Nramp (natural resistance-associated macrophage protein) is involved in iron transport, and deficiency may be important in NTS and other intracellular infections [181]. However, as yet there is no information on Nramp expression in HIV infection.

Conclusions

There is a complex interplay of bacterial virulence mechanisms and host immunity which determines the severity of NTS disease. In adult HIV infection and in paediatric malarial anaemia, sickle cell disease and malnutrition, the balance is tilted in favour of the bacterium. Further understanding of the epidemiology of transmission and of the immune mechanisms involved in these groups will greatly increase our understanding of the pathogenesis of invasive NTS disease.

DRUG INTERACTIONS IN AIDS

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Antiretroviral treatment for HIV involves a combination of nucleoside analogue reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors and protease inhibitors [182] (Table 6). However, the widespread use of other drugs (e.g., antiviral, antifungal and antimicrobial agents) administered concomitantly with antiretroviral treatment provides an enormous potential for drug–drug interactions [183].

It is beyond the capacity of this review to describe in detail all the potential drug interactions with antiretroviral agents used to treat HIV disease; therefore, it will focus on some of the major drug interactions. For those involved in prescribing for HIV positive patients we have set up a website with comprehensive drug–drug interaction charts which present an up-to-date evaluation of all potential interactions with antiretroviral treatment [184].

Nucleoside reverse transcriptase inhibitors

The six nucleoside reverse transcriptase inhibitors (NRTIs) listed in Table 6 are analogues of the host's deoxynucleosides. NRTIs have a short plasma elimination half-life and are predominantly eliminated by the kidneys [185, 186]. Zidovudine (ZDV) is the only NRTI to undergo significant hepatic metabolism (to its glucuronide metabolite) [187]. Therefore, drugs inhibiting glucuronidation (e.g., probenecid) are likely to increase the plasma concentration of ZDV [188]. Interaction studies with other antiretroviral therapies and NRTIs have demonstrated only modest changes in the area under the plasma drug concentration–time curves (AUCs) of the NRTIs [189]. These interactions are unlikely to be clinically relevant because activity depends on the extent of intracellular phosphorylation [190].

NRTIs must be taken up into target cells and phosphorylated to their active triphosphate analogues by intracellular enzymes [191, 192]. The active 5'-triphosphates (ddNTPs) compete with endogenous deoxynucleoside triphosphates (dNTPs) for incorporation into viral DNA, thus inhibiting viral DNA synthesis [193]. Phosphorylation of the NRTIs is catalysed by enzymes that are normally responsible

for the formation of deoxynucleosides through the salvage pathway within the host cell (Fig. 4) [193].

Maintenance of an adequate concentration of ddNTP (and the ratio of ddNTP to endogenous dNTP) is essential for successful therapy. Relatively few interactions have been documented associated with intracellular phosphorylation. However, an important interaction occurs between the thymidine analogues zidovudine (ZDV) and stavudine (d4T) which compete for the host cell's intracellular kinases (Fig. 4). Co-administration of ZDV and d4T at equal concentrations *in vitro* markedly decreases d4T activation, but ZDV phosphorylation is unaltered [194]. This is because thymidine kinase has a 600-fold higher affinity for ZDV than d4T. The clinical effect of this interaction was seen in ACTG 290, when the ZDV plus d4T arm was discontinued after interim analysis demonstrated that subjects receiving this combination had significantly greater decreases in CD4 counts than the other arms [195].

The two cytidine analogues also compete for activating enzymes (Fig. 4). Zalcitabine (ddC) inhibits lamivudine (3TC) phosphorylation *in vitro* at higher concentrations than observed *in vivo*, but ddC activation is reduced by 3TC at similar concentration ratios to those seen in patients [196, 197].

Modulation of NRTI phosphorylation is now a realistic therapeutic strategy. Hydroxyurea inhibits ribonucleotide reductase, which results in decreased endogenous dNTPs [198], an increase in the ddNTP/dNTP ratio and thus anti-HIV activity. Clinical benefit has been demonstrated clinically when hydroxyurea was combined with didanosine [199]. It is clearly important to measure both ddNTP and dNTP in intracellular pharmacokinetic studies. A knowledge of the mechanism of action of NRTI may allow clinical benefit from drug interactions.

Drug interactions involving ddI occur as a consequence of the neutralising agents in oral preparations of the drug. Therefore, it is recommended that ddI is administered 1 h apart from drugs requiring an acidic environment for optimal absorption (e.g., indinavir) [200].

Protease inhibitors

Protease inhibitors are extensively metabolised by enterocyte and hepatic cytochrome P450 (CYP) enzymes [201]. The iso-enzyme largely responsible for metabolism of the protease inhibitors is cytochrome P450 3A4, with CYP2C9 and CYP2D6 also contributing [189]. In addition to being metabolised by this enzyme, each protease inhibitor can induce or inhibit CYP3A and other CYP enzymes, or both. This results in a complex set of drug interactions (Table 7).

Table 6. Currently available antiretroviral therapies [182]

Nucleoside analogues	Protease inhibitors	Non-nucleoside reverse transcriptase inhibitors
Zidovudine (ZDV)	Saquinavir (SQV)	Nevirapine
Zalcitabine (ddC)	Ritonavir (RTV)	Delavirdine
Didanosine (ddI)	Indinavir (IDV)	Efavirenz
Stavudine (d4T)	Nelfinavir (NFV)	
Lamivudine (3TC)	Ampranavir (APV)	
Abacavir (ABC)

Fig. 4. Activation of the nucleoside analogues:1592, abacavir; CBV, carbovir.

Table 7. Summary of the pharmacokinetic interactions between protease inhibitors and non-nucleoside reverse transcriptase inhibitors

Affecting drugs	Affected drugs				
	Indinavir	Ritonavir	Saquinavir (soft gel)	Nelfinavir	Amprenavir
Indinavir	...	No interaction	↑ 600%	↑ 84%	↑ 64%
Ritonavir	↑ 500%	...	↑ 2000%	↑ 250%	No data
Saquinavir	No data	No interaction	...	↑ 17 %	↓ 36%
Nelfinavir	↑ 51%	No interaction	↑ 500%	...	↑ C _{min} 167%
Amprenavir	↓ 38%	No data	↓ 18%	↑ 15%	...
Nevirapine	↓ 28%	↓ 10%	↓ 27%	No interaction	No data
Delavirdine	↑ C _{max} 2–500%	↑ 70%	↑ 500%	↑ 90%	No data
Efavirenz	↓ C _{max} 35%	↑ 17%	No data	↑ 15%	↓ 36%

Interactions are expressed as a percentage change in the area under the concentration-time curve (AUC) unless stated. C_{max} and C_{min}, maximum and minimum plasma concentration; ↑ and ↓, increase and decrease (for references see text).

Saquinavir (SQV)

The poor systemic availability of SQV (hard gel capsule *c.* 4%; soft gel capsule *c.* 12%) is a consequence of rapid and extensive metabolism [202, 203]. SQV is metabolised by CYP3A4 and so has the potential to interact with drugs that either induce or inhibit this enzyme [201]. Induction of SQV is particularly important as many patients are believed to have SQV plasma levels near or below the minimum effective concentrations [204]. Co-administration of inducers of CYP 3A4 increase metabolism resulting in decreases in steady-state AUC and peak plasma concentration (C_{max}) of SQV. In contrast, drugs that inhibit CYP3A4 may increase plasma concentrations of SQV, increasing the AUC of SQV.

Ritonavir is a potent inhibitor of CYP3A4 and co-administration of RTV and SQV results in increases in the C_{max} and AUC of SQV [205, 206]. The AUCs of the hard and soft gel formulations of SQV have been shown to increase in the presence of RTV >50-fold and 20-fold respectively [206, 207]. Similarly, the protease inhibitors nelfinavir and indinavir inhibit CYP3A4, increasing mean plasma concentrations and AUCs of SQV during co-administration. Indinavir and nelfinavir increased the AUC of soft gel SQV by six-fold and five-fold respectively [207, 208]. These interactions are of clinical benefit, as increased bioavailability results in enhanced antiviral activity and, perhaps, dose reduction.

The non-nucleoside nevirapine is an inducer of CYP3A4 enzymes and has been shown to reduce the AUC of hard gel SQV by 27% [209, 210]. However, administration of hard gel SQV with the CYP3A inhibitor, delavirdine, results in a five-fold increase in the AUC of SQV [211]. Drugs used to treat opportunist infections such as clarithromycin, erythromycin, fluconazole and ketoconazole also inhibit CYP3A4 [207]. Concomitant administration of SQV (soft gel) with clarithromycin resulted in increases in mean SQV C_{max} and AUC of 187 and 177%, respectively [207].

SQV itself is a weak inhibitor of CYP3A4 and increases the plasma concentrations of drugs also metabolised by this isozyme [212]. However, SQV is the least potent inhibitor of CYP3A4 and has little effect on the pharmacokinetics of the other protease inhibitors (Table 7). Co-administration of SQV and terfenadine resulted in increases in terfenadine C_{max} (253%) and AUC (368%) [213]. The resulting risk of serious cardiac arrhythmias means that these drugs should not be given together.

The role of P-glycoprotein is also a possible mechanism for drug interactions. P-glycoprotein is a transmembrane protein capable of actively transporting drugs out of intestinal, hepatic and renal cells, thus increasing cellular clearance [214]. P-glycoprotein and CYP3A metabolism have overlapping substrate specificities and SQV has been shown to be a substrate for P-glycoprotein [215]. Drug interactions may be anticipated with either substrates or inhibitors of P-glycoprotein (e.g., ketoconazole) [216].

Ritonavir (RTV)

RTV is a potent inhibitor of CYP3A4 and to a lesser extent CYP2D6 and CYP2C9 [205]. RTV increases the plasma concentration of agents metabolised by CYP3A4 but to a greater extent than SQV. Giving a combination of RTV and IDV to healthy volunteers resulted in increases in the AUC (480%) and C_{max} (110%) of IDV [217]. Similarly, co-administration of NFV and RTV produced a 152% increase in the AUC_{0-8h} of NFV [218]. However, the plasma concentration of RTV is unaltered with IDV, NFV and SQV [217, 219, 220].

The potent inhibitory effect of RTV on CYP3A4 activity means that concomitant use with astimazole, terfenadine and cisapride is likely to be associated with cardiac arrhythmias [221]. Concurrent use of RTV with a number of anti-arrhythmics and sedatives is contra-indicated.

RTV enhances the bioavailability of drugs metabolised by CYP3A [221]. Agents metabolised by other CYP450s may have reduced bioavailability. Inducers of CYP3A4 are likely to lead to sub-therapeutic levels of RTV and should be avoided. RTV induces its own metabolism and also induces the metabolism of some other drugs.

Indinavir (IDV)

Inhibition of CYP3A4 by IDV leads to interactions with substrates of this enzyme [222]. Also, addition of an inhibitor of CYP3A4 to an IDV regimen leads to an increase in IDV concentrations. On the other hand, patients treated with inducers of this enzyme, such as phenobarbital or rifabutin, result in decreased IDV concentrations [204].

Drug interactions with dual PI therapy are often beneficial. A study that used lower dose RTV (100 mg) in combination with higher doses of IDV showed enhanced IDV concentrations with increased trough and decreased peak levels [223]. This may reduce toxicity whilst sustaining efficacy.

The non-nucleoside reverse transcriptase inhibitors nevirapine and efavirenz are enzyme inducers [224]. Both nevirapine and efavirenz decreased IDV levels by *c.* 30%. In contrast, delavirdine is an inhibitor of CYP3A4 and has been shown to increase the AUC of indinavir [211].

Co-administration of IDV and nelfinavir in healthy volunteers resulted in increases in the AUC of both IDV (51%) and nelfinavir (84%). Similar AUC increases in both IDV (38%) and amprenavir (64%) were seen in HIV patients [225].

Nelfinavir (NFV)

NFV has potential to impair drug metabolism, but drug interactions are further complicated by the fact that NFV has an active metabolite (AG-1402) [226,227]. In one study delavirdine increased the AUC of nelfinavir by 90% but decreased AG-1402 by 44%, thereby prolonging the overall elimination half-life [228].

Amprenavir (APV)

APV has been shown to inhibit CYP3A4 and CYP2C9 with inhibition of 3A4 similar to NFV and IDV [229]. Interactions between APV and SQV or NFV are modest and unlikely to be clinically significant [229]. Co-administration of APV (1200 mg) with ketoconazole (400 mg) resulted in *c.* 40% increases in the AUCs of both agents. Clinically significant interactions have been demonstrated with rifabutin and rifampicin and it is recommended that APV should not be administered with rifampicin [229]. Induction by the non-nucleoside reverse transcriptase inhibitor, efavirenz, results in a

36% decrease in the AUC of amprenavir when these two drugs are administered [230].

Non-nucleoside reverse transcriptase inhibitors

The non-nucleoside reverse transcriptase inhibitors currently available – nevirapine, delavirdine and efavirenz – undergo extensive metabolism [209]. Drug–drug interactions are anticipated when these drugs are administered with protease inhibitors or if two non-nucleoside reverse transcriptase inhibitors are prescribed.

Nevirapine is an inducer of cytochrome P450 enzymes which reduces the AUCs of the protease inhibitors. Nevirapine also reduced the clarithromycin AUC by 30%. However, the antibacterial active metabolite 14-OH clarithromycin was increased, probably due to induction of CYP450 metabolism by nevirapine [231].

NFV decreased delavirdine AUC (42%) in healthy volunteers, but no data are available for HIV patients [228]. None of the other protease inhibitors studied (SQV, RTV and IDV) have shown any effect on delavirdine metabolism [232–234]. Surprisingly, delavirdine concentrations decrease in the presence of adefovir. Delavirdine is metabolised in the liver whereas adefovir is excreted renally. The reason for the interaction in this small study is unknown but may be an effect of adefovir on the P-glycoprotein drug transporter.

Co-administration of didanosine and delavirdine produced a decrease in the AUC of delavirdine (38%) compared with the AUC when administration of the two drugs was separated by 1 h [235]. This is the only reported drug–drug interaction between nucleoside analogue and non-nucleoside analogue reverse transcriptase inhibitors.

The induction effect of efavirenz is not as predictable as with nevirapine. Combination of nelfinavir and efavirenz in healthy volunteers resulted in an inhibitory effect with an increase in the AUC by 20% and a decrease in the AUC of its metabolite AG-1402 by 37% [236]. The only protease inhibitor to alter the pharmacokinetics of efavirenz was ritonavir, which increased the plasma concentration of efavirenz by 21%, but this is unlikely to require dose modification [237].

Conclusion

This review demonstrates that drug reactions may compromise therapeutic efficacy or increase toxicity. However, some of the interactions between two-drug combinations of protease inhibitors are beneficial. It is essential that clinicians have a full understanding of

drug interactions when prescribing to HIV patients. A comprehensive updated summary of the possible drug interactions encountered with antiretroviral therapy is available through the internet [184].

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