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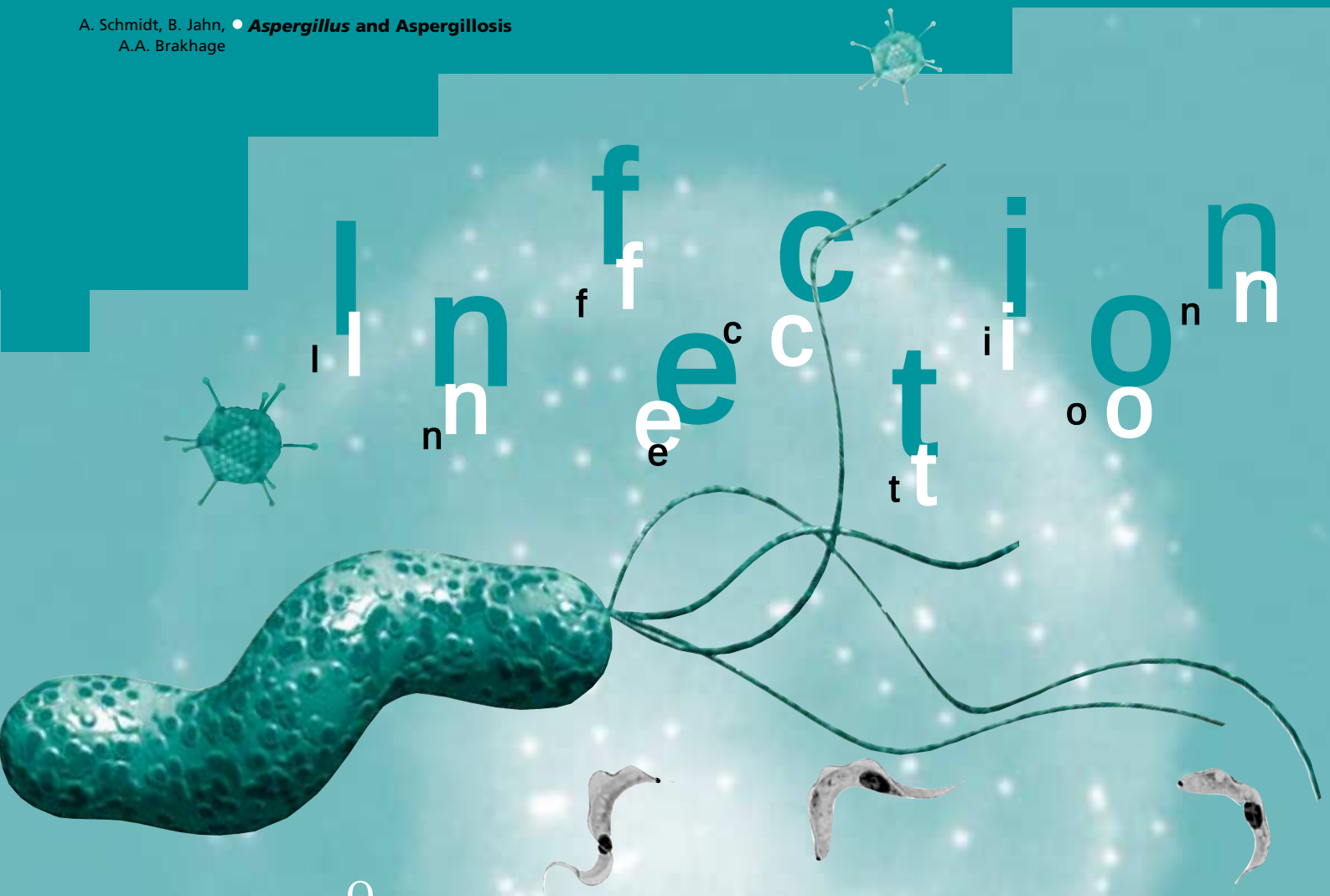

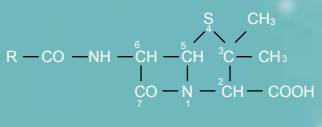

Only some decades ago, it was widely believed that science would triumph over infectious diseases. Powerful antibiotics, vaccines and improved sanitation had facilitated the control of many diseases like pneumonia or polio, particularly in industrialized countries. This success bred a certain attitude of complacency, as nobody anticipated the extraordinary resilience of microbes and their ability to evolve and adapt. But infectious diseases are still the leading cause of death worldwide. In fact, the 1980s and 1990s have seen the emergence of new diseases such as AIDS, Lyme disease or Hantavirus pulmonary syndrome, while old diseases such as tuberculosis and malaria are on the rise again. The reasons for this are manifold: global travel, population shifts, urbanization, ecological changes, inadequate public health measures, changes in human behavior, and most importantly, increasing microbial drug resistance.

Infection pervades almost every medical discipline, and more and more microbes are being identified as causative agents of chronic inflammatory disorders or cancers (e.g. *Helicobacter pylori* as the cause of peptic ulcers and gastric cancer, papillomaviruses which are linked to cervical cancer, and a new human herpesvirus (KSHV) which is involved in the pathogenesis of Kaposi's sarcoma).

The world of infectious agents is immensely diverse, ranging from prion proteins to helminths. Aiming to illustrate this diversity, this issue of the *Karger Gazette* features articles on four examples of new or re-emerging infectious agents, each representing a different group. Gastritis, peptic ulcers and certain gastric cancers caused by infection with the bacterium *Helicobacter pylori* affect millions of people worldwide. These diseases which were previously considered chronic are now readily curable with antibiotics. A report on sleeping sickness, an 'old', almost forgotten disease caused by trypanosome parasites, provides a good example of how an infectious disease once well controlled can flare up due to declining health infrastructures, lack of funding, and political unrest. Prions, the most recently discovered group of infectious agents, are discussed in the third article: they have challenged the established concepts of infection and have been the topic of much scientific and political debate following the recent outbreak of BSE in England, as nobody can yet predict what impact they will have in years to come. And finally, the fungus *Aspergillus* and its pathogenic impact are elucidated – an agent which has become a major health problem in recent years as a cause of opportunistic, often lethal infections in immunocompromised people. These four articles represent only a small fraction of the diversity of infections. We hope they will provide interesting reading and at the same time illustrate the imminent threat to human health from new infections and antimicrobial resistance which will most certainly intensify in the 21st century.

Helicobacter pylori Infection

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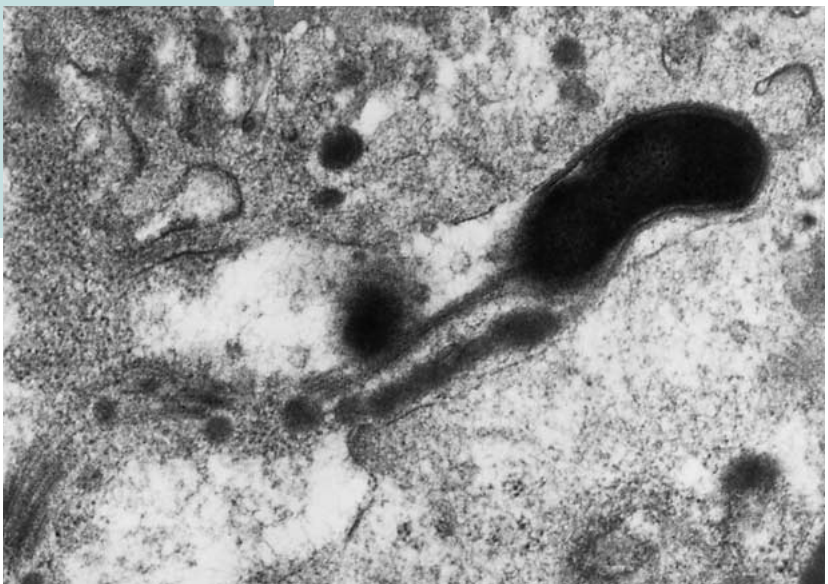
Helicobacter pylori is a spiral or curved gram-negative bacterium which colonizes the surface of gastric epithelial cells. Persistent colonization always causes chronic active gastritis. This *H. pylori*-associated gastritis is recognized as a major pathogenic factor in benign and malignant gastroduodenal disease. Around 20% of infected individuals progress to peptic ulcer disease, others to chronic atrophic gastritis and gastric adenocarcinoma, while a smaller number may develop lymphatic neoplasia. The potential to cure *H. pylori* infection, i.e. to eradicate *H. pylori*, has revolutionized the therapeutic concept of peptic ulcer disease, providing new hope for patients suffering from what used to be considered a chronic disease. Moreover, numerous scientific data suggest that eradication of *H. pylori* could be beneficial for the prophylaxis of gastric malignancies as well as for curing manifest low-malignant gastric MALT lymphoma (mucosa-associated lymphoid tissue lymphoma). Lately, the broadening of indications for curing *H. pylori* infection has provoked lively scientific discussion as to whether widespread or even global eradication of *H. pylori* is possible or desirable.

Fig. 1. Electron microscopy of a *H. pylori* depicting two features essential for colonization of gastric mucosa: (1) motility, provided by unipolar flagellae, and (2) adherence, the close contact of *H. pylori* to the cell membrane of gastric epithelial cells.



The *H. pylori* Story

The history of the detection of *H. pylori* is a prime example of how a medical dogma can lead to scientific observations being neglected. This dogma maintained that the stomach is sterile due to its gastric acid content. Another dictum which governed former pathophysiological concepts was that of K. Schwartz, who stated in 1910 that ulcers occur when too much acid damages the gastric mucosa ('no acid, no ulcer').

Nevertheless, evidence for bacterial involvement had been found as early as the latter half of the 19th century. Bacteria in the human stomach had already been reported by G. Böttcher in 1874. In 1893, G. Bizzozero described spiral organisms in the stomach of dogs, and in 1906, W. Krienitz found 'spirochaetes' in the stomach of a patient with stomach cancer. J.M. Luck and T.N. Seth isolated the enzyme urease from gastric mucus in 1924. In a larger series in 1939, J.L. Doenges found bacterial colonization of the gastric mucosa in 43% of the patients. But it was not until 1979 that the pathologist J. Robin Warren again observed curved or spiral bacteria in inflamed gastric mucosa and encouraged the internist Barry Marshall to try and cultivate these bacteria. Marshall was only successful after leaving the cultures incubated for an unintended long period of more than 4 days over a holiday in 1983 [1]. Warren and Marshall also discovered the close link between *H. pylori* and gastritis and peptic ulcer disease. By swallowing a suspension of *H. pylori*, Marshall induced gastritis in his own stomach, from which the suspected pathogen could be re-isolated, thereby fulfilling the postulates of Robert Koch for proof of an infectious disease. Initially named *Campylobacter pyloridis*, the bacterium was reclassified in 1989 based on RNA analysis: the species is now called *Helicobacter pylori* and belongs to the newly discovered genus of *Helicobacter*.

Microbiology

It is now recognized that different species of *Helicobacter* are widespread in mammalian stomachs. Furthermore, certain *Helicobacter* species which are not adapted to an acid milieu have been found in the gut and the gallbladder, but in a much lower density than in the stomach. An association with gallbladder carcinoma in South America is currently being discussed .

Helicobacter heilmannii, characterized histologically by a longer rod than *H. pylori* and by its corkscrew shape, also colonizes the human gastric mucosa, but is found with a prevalence of less than 0.3% in gastric biopsies. It is mainly acquired from animals, generally causes a gastritis of lesser intensity, and is only seldom linked to peptic ulcer or gastric cancer, but perhaps to gastric MALT lymphoma.

In vitro, *H. pylori* grows slowly and needs fastidious culture conditions with blood or serum supplements and a microaerophilic atmosphere. Minor differences in culture conditions may explain the interlaboratory variability of in vitro testing for microbial resistance. Standardization is now intended, at least at national levels. The best results are achieved with the agar dilution test, but the elipsometer test is also adequate and cheaper.

H. pylori as a Pathogen

Colonization

The most important morphological and biochemical features of *H. pylori*, essential for the colonization of the acidic milieu of the gastric mucosa, are:

1. Urease, an enzyme that hydrolizes endogenous urea to ammonia and carbon dioxide. Ammonia neutralizes the acid in the immediate microenvironment.

2. Motility, provided by the bacteria's spiral shape and sheeted unipolar flagella, the motor for its rapid movement through the viscous mucus layer.

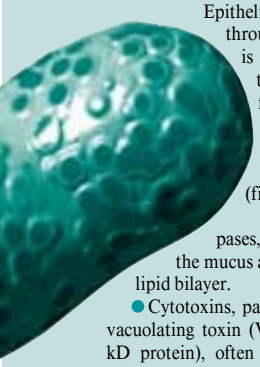
3. Adhesins, providing close contact to gastric epithelial cells (fig. 1). This adhesion is specific to gastric epithelial cells, as *H. pylori* can colonize ectopic or metaplastic gastric epithelium outside the stomach, but not any other cells.

Gastritis

The detection of an infective cause of chronic gastritis led to a new classification of gastritis, agreed upon by an international panel of pathologists in 1990 in Sydney and updated in 1994 in Houston [2]. The initial, often clinically unrecognized *H. pylori* infection results in acute gastritis which is typically followed within a few days by chronic active gastritis (fig. 2); 'chronic' refers to lymphocyte infiltration, 'active' to infiltration by polymorphonuclear leukocytes. The grade of inflammation as well as the distribution of the gastritis between antrum and corpus vary greatly between individuals. Bacterial, host and environmental factors contribute to the different expressions of gastritis, although the determinants and their interference are still incompletely understood. The distribution of gastritis is one of the few factors which correlate with *H. pylori*-associated disease, as outlined below. A clinically relevant prediction, however, as to which individual infected with *H. pylori* will develop duodenal or gastric ulcer or gastric malignancy, is currently not possible based on parameters of gastritis alone.

There is, however, a close link between the amount of acid secreted and the distribution of gastritis. Acid secretion is normal or increased in antrum-predominant gastritis, whereas it is reduced in corpus-predominant gastritis. Which of the two pathogenetic factors, distribution of gastritis or acid secretion, precedes and determines the other remains an unresolved issue. Thus, reducing acid secretion, e.g. by treatment with antisecretory agents, leads to a higher grade of gastritis in the corpus and fundus. On the other hand, corpus-predominant gastritis confers reduced acid secretion and even

corpus gland atrophy. Recently, autoantibodies targeted against parietal cells were found to be induced by *H. pylori* gastritis.



Epithelial damage through *H. pylori* is mediated by the following factors, currently considered most important (fig. 3):

- Phospholipases, degrading the mucus and phospholipid bilayer.
- Cytotoxins, particularly the vacuolating toxin (VacA, an 87-kD protein), often co-expressed with CagA, an immunodominant 120- to 160-kD protein, the function of which is not known exactly. Some strains of *H. pylori* have a higher virulence, which is defined by the presence of the *cagA* gene product (and more recently by a group of genes around *cagA* known as pathogenicity islet); these strains are more frequently associated with severe gastritis, atrophic gastritis, peptic ulcer and, possibly, gastric carcinoma.
- Ammonia, exerting a cytotoxic effect by increasing epithelial permeability to hydrogen ions.
- Release of lysosomal enzymes, free oxygen radicals, cytokines and platelet-activating factor (PAF) from neutrophils and macrophages in the gastric mucosa.
- Activation of the complement system and PAF through antigen-antibody complexes following activation of T and B lymphocytes.

Peptic Ulcer

H. pylori is present in 90–95% of patients with duodenal ulcer. Although the prevalence of *H. pylori* infection in patients with gastric ulcer is somewhat lower and more variable (60–80%), this figure approaches 100% when specific etiologies such as nonsteroidal anti-inflammatory drug (NSAID) therapy and Zollinger-Ellison syndrome are excluded. The most compelling evidence for a causal relationship between *H. pylori* and peptic ulcer disease is the dramatic reduction in ulcer recurrence and complication following successful eradication of the microorganism.

Duodenal ulcer is mainly linked to antrum-predominant gastritis. Duodenal ulcer patients often differ from asymptomatic individuals infected with *H. pylori* by their increased acid secretion, which persists after eradication. Hence, one of the pathomechanisms of duodenal ulcer is acid hypersecretion, causing gastric metaplasia of the duodenum which in turn is then colonized and damaged by *H. pylori.*

The distribution of gastritis in gastric ulcer is mostly balanced between antrum and corpus with acid secretion being normal or slightly reduced. This emphasizes the capacity of *H. pylori* to damage the epithelium even when acid secretion is reduced.

Gastric Cancer

In 1994, the International Agency for Research on Cancer concluded that, based on evidence derived mainly from epidemiological studies, *H. pylori* infection is a risk factor for gastric cancer. To date, it is widely accepted that nonatrophic gastritis caused by *H. pylori* may represent the first step in a sequence of events leading to gastric carcinoma [3]. This process starts with superficial gastritis, progresses to atrophic gastritis and then to intestinal metaplasia. In some cases, this evolution continues to dysplasia and finally gastric carcinoma.

Intestinal metaplasia is a critical step in the potential evolution of gastric malignancy; it appears as a consequence of long-term *H. pylori* infection and is concomitant with atrophic gastritis. Some authors consider intestinal metaplasia as a surrogate marker rather than as a biological precursor of malignancy. A number of factors are associated with intestinal metaplasia, such as bile reflux, low-vitamin diet, increasing age, and heredity. Some data support the reversibility of intestinal metaplasia after *H. pylori* eradication. Another important risk factor for gastric cancer is a higher grade and more active gastritis in the corpus versus the antrum. As a consequence, acid secretion is reduced, leading to increased levels of mutagenic and carcinogenic N-nitroso compounds. However, till now there has been no definitive proof of the link between *H. pylori* and gastric cancer. Only prospective interventional studies will be able to show whether a large number of gastric cancers can be prevented by curing *H. pylori* infection.

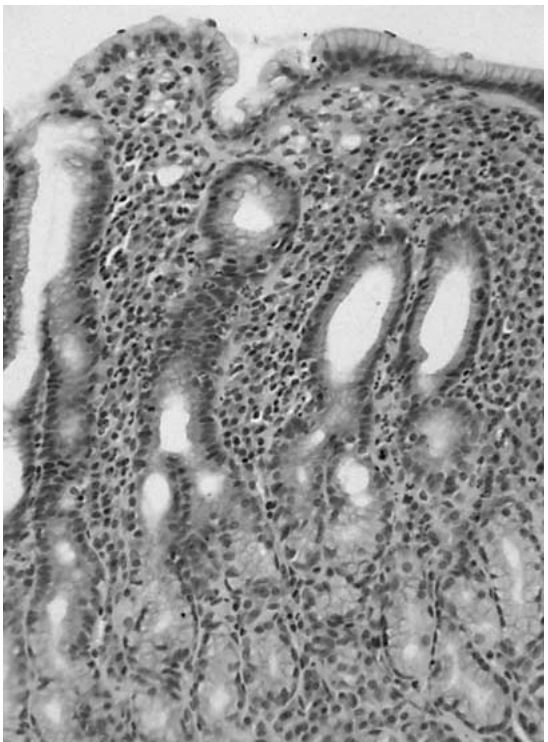


Fig. 2. Histology of gastric mucosa with chronic active gastritis, infiltration with lymphocytes and polymorphonuclear cells. HE. Courtesy of Dr. T. Günther, Institute of Pathology, Magdeburg.

Gastric MALT Lymphoma

Mucosa-associated lymphoid tissue (MALT), which is not found in normal gastric mucosa, develops in response to chronic *H. pylori* infection. It is from this tissue that MALT lymphoma may arise. 95% of all gastric MALT lymphomas are associated with *H. pylori* gastritis. Gastric lymphoma is less frequent than carcinoma, with a frequency ratio of 1:20. Dense infiltration of the gastric mucosa with MALT indicates a risk for MALT lymphoma, while MALT lymphoma is not correlated to other gastritis parameters or bacterial virulence factors. Eradication of *H. pylori* led to longstanding remission of low-grade MALT lymphoma stage EI1 in about 60% of the cases.

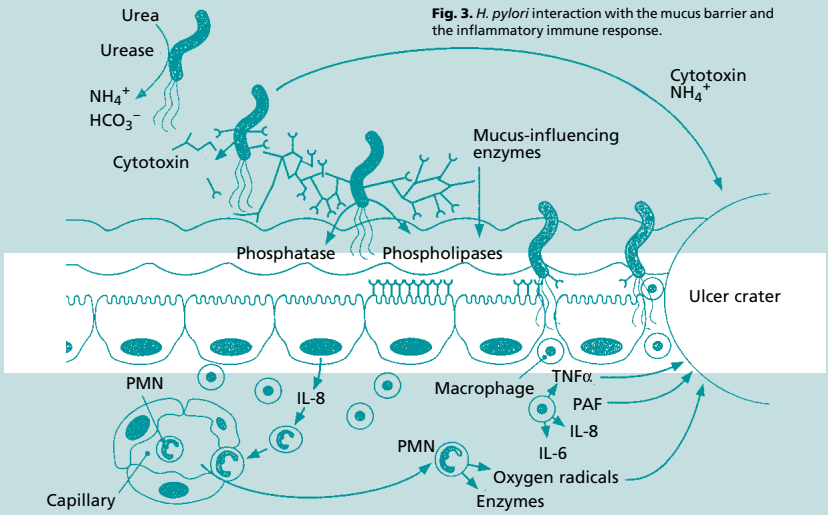


Fig. 3. *H. pylori* interaction with the mucus barrier and the inflammatory immune response.

Epidemiology and Transmission

The prevalence of *H. pylori* infection is related to age, geographic area and socioeconomic status. The infection is mostly acquired during childhood. In developed countries, the age-dependent prevalence roughly parallels the age expressed in years, e.g. 30% in 30-year-old subjects. This age-dependent increase in prevalence is mainly due to a cohort effect rather than to newly acquired infections. The cohort effect means that older individuals carry the infection more frequently because they were more likely to have been infected during their childhood when hygiene and living conditions were not so good. In developed countries, the annual rate of real new infections in adulthood is only 0.3%. The total prevalence, independent of age, has been declining since the beginning of the 20th century. In contrast, in the developing world, *H. pylori* infection is still very frequent in all age classes. 50% of the children are infected by the age of 10. Irrespective of geographic origin, the prevalence is higher in poorer more crowded households and in environments with poorer sanitary facilities.

The route of transmission of *H. pylori* infection is oral-oral or fecal-oral. Evidence of oral-oral transmission is provided by documented cases of infection arising from the use of gastric aspiration tubes and contaminated endoscopes. The pattern of acquisition of *H. pylori* infection in developing countries reflects that of hepatitis A, suggesting that transmission also occurs via the fecal-oral route. This is supported by the fact that *H. pylori* cultures can survive in water for periods of up to 48 h, although there are inconsistent findings regarding the isolation of the organism from human feces.

Table 1. Indications for *H. pylori* eradication: Consensus Report of the European Helicobacter Pylori Study Group 1997

Indications for *H. pylori* eradication therapy	Strength of supporting evidence
Strongly recommended	
Peptic ulcer disease	unequivocal
Bleeding peptic ulcer	unequivocal
Low-grade gastric MALT lymphoma	unequivocal
Gastritis with severe abnormalities	supportive
Following early resection for gastric cancer	supportive
Advisable	
Functional dyspepsia, after full investigation	equivocal
Family history of gastric cancer	equivocal
Long-term treatment with PPI for gastroesophageal reflux disease	supportive
Planned or existing NSAID therapy	equivocal
Following gastric surgery for peptic ulcer	supportive
Patient´s wishes	equivocal
Uncertain	
Prevention of gastric cancer in the absence of risk factors	equivocal
Asymptomatic subjects	equivocal
Extra-alimentary tract disease	equivocal

Table 2. Three options as standard therapy for *H. pylori* eradication, each given for 1 week

	Clarithromycin	Metronidazole (or tinidazole)	Amoxicillin
PPI	250 mg b.i.d	400 mg b.i.d	
PPI	500 mg b.i.d		1,000 mg b.i.d
PPI		400 mg t.i.d	500 mg t.i.d

PPI = Proton pump inhibitor, twice daily standard dosis [4].

Diagnosis: Indications and Methods

As the outcome of *H. pylori* infection can vary from no symptoms in some individuals to severe disease in others, a diagnosis should only be intended if treatment is considered. The indications for *H. pylori* eradication were worked out by the European Helicobacter Pylori Study Group [4] at a consensus conference in 1996 (table 1). It has been a very controversial and unresolved issue since 1996, whether, in the long term, *H. pylori* eradication may trigger gastroesophageal reflux disease [5]. This would render questionable the indication for *H. pylori* eradication before long-term proton pump inhibitor (PPI) therapy.

Endoscopic methods for diagnosis of *H. pylori* infection require mucosal biopsies from the gastric antrum and, especially when following treatment with an antibiotic or PPI, the gastric fundus. Tests include rapid urease testing, histology, bacterial culture, and polymerase chain reaction (PCR). Rapid urease testing was introduced by Marshall. By a simple pH-dependent color reaction, it indicates the urease activity of *H. pylori* in the biopsy. The rapid urease test is the most useful in routine practice in the presence of duodenal ulcer since, in addition to its moderate-to-high sensitivity (90%) and high specificity (95%) for *H. pylori*, it is relatively cheap, easy to perform, observer-independent and provides quick results. The sensitivity may, however, be reduced as the result of a reduction in bacterial load following antibiotic or PPI treatment, increasing the risk of a false-negative result.

Histology also has a high sensitivity (85–90%) and specificity (93–100%) for *H. pylori*, and the added advantage of being able to demonstrate chronic active gastritis and other mucosal abnormalities. In the absence of histological signs of chronic mucosal inflammation (e.g. PMN leukocyte infiltration), *H. pylori* infection can be excluded. The drawbacks include high cost and potential for interobserver variability in interpreting test results.

Although bacterial culture is widely regarded as the gold standard for the diagnosis of *H. pylori*, the procedure is expensive, technically demanding and long. For these reasons, it is generally reserved for determining antibacterial sensitivity following unsuccessful *H. pylori* eradication therapy.

PCR achieves high (95%) specificity and sensitivity for *H. pylori*, but is expensive and currently used only as a research tool.

Nonendoscopic diagnostic methods include antibody detection using an ELISA or agglutination, and the urea breath test. Serology has the advantage over the urea breath test of being simple, inexpensive and widely available. However, it is also imperative that these tests are validated in different populations as the accuracy of one and the same test is variable in different geographic areas. For monitoring the success of eradication therapy, serology is unsuitable because of the slow decline in *H. pylori*-specific IgG and IgA antibody titres.

In contrast, the urea breath test produces a positive result only in the setting of current infection and is therefore the most appropriate nonendoscopic method for confirming successful *H. pylori* eradication. The currently available carbon-radiolabelled urea breath tests – the radioactive ^{14}C test and the nonradioactive ^{13}C test – are both highly sensitive (90–100%) and specific (95–100%), but are relatively expensive. However, equipment for the ^{13}C test is becoming more readily available and widely distributed.

The choice of diagnostic test for assessing *H. pylori* status is dictated by clinical considerations and the need for endoscopy.

Treating *H. pylori* Infection

Evolution of *H. pylori* Treatment

Numerous treatment regimens have been investigated for the eradication of *H. pylori* infection, predominantly in patients with duodenal ulceration. Nowadays, highly efficacious and safe standard combination treatments are available. But for the rare cases of treatment failure, the decision for a second-line treatment is often difficult as the factors leading to failure are complex. The rules for *H. pylori* treatment are mainly based on clinical trials, as microbial in vitro tests regarding antibiotic activity are only poorly transferable to clinical treatment success.

Antibacterial monotherapy should be discouraged in the eradication of *H. pylori*, since such regimens typically achieve cure rates of < 20% after 2–4 weeks of therapy and involve a high risk of antibacterial resistance. Until recently, the standard approach to *H. pylori* eradication was a 14-day course of bismuth-based triple therapy, comprising colloidal bismuth, a nitroimidazole (typically metronidazole) and either tetracycline or amoxicillin. Although *H. pylori* eradication rates of 80–90% have been obtained in clinical trials of bismuth triple therapy, the success of these regimens in community use is limited by poor tolerability, poor patient compliance with the complex dosage regimen, and a loss of efficacy because of pretherapeutic microbial resistance against nitroimidazoles. Furthermore, in patients with active disease, it is often necessary to add an antisecretory agent to provide rapid symptom relief and promote ulcer healing. These disadvantages of bismuth-based triple therapy could be overcome by PPI-based dual therapies, containing amoxicillin or clarithromycin in combination with a PPI. But the *H. pylori* cure rates of PPI-based dual therapies turned out to be highly variable: therefore, these treatments were favored only during the period 1991–1995 in some countries.

Standard Treatment of *H. pylori*

The current standard was achieved by adding a second antibacterial agent to dual PPI-based regimens. The first experiments with 1-week triple therapies were performed in 1993 (PPI-amoxicillin-metronidazole) and 1994 (PPI-clarithromycin-tinidazole), followed by large-scale randomized, controlled multicenter studies. In most countries, the approved treatments encompass 1-week PPI-based triple therapies with two of the following three antibiotics: clarithromycin, a nitroimidazole (metronidazole or tinidazole) or amoxicillin. The dosages and schedules of the mentioned antibiotics differ between the three possible combinations (table 2). The highest *H. pylori* cure rates are consistently achieved with the first two mentioned antibiotic combinations, i.e. over 90% in 'per protocol' analysis and over 80% in ' intention to treat' analysis.

Factors Leading to Treatment Failure and Selection of Second-Line Treatment

The main factors known to lead to treatment failure are insufficient compliance and pre-existent microbial resistance [6]. Side effects of 1-week PPI triple therapies occur with frequencies between 10 and 30%, but are generally mild and lead only rarely to discontinuation of treatment. Loose stools and diarrhea are experienced more frequently with clarithromycin-amoxicillin than with clarithromycin-nitroimidazole. The focus nowadays is on antibiotic resistance. Pretherapeutic in vitro resistance against clarithromycin is linked to treatment failure in about half the cases with both clarithromycin-containing regimens, whereas in nitroimidazole-resistant strains, the *H. pylori* cure rates of PPI-clarithromycin-metronidazole are reduced by only 10–20%. The negative impact of nitronidazole resistance on PPI-amoxicillin-nitroimidazole is another 10% higher. The prevalence of pretherapeutic clarithromycin resistance amounts to 2–3% in Northern Europe, 5–10% in American and Asian countries, and up to 15% in Southern Europe. The prevalence of metronidazole resistance is much higher and more variable, ranging from 15 to 60% in developed and up to 90% in developing countries. Resistance against penicillins was thought nonexistent for several years. After initial reports on single cases in 1997 in the Netherlands, Sardinia and the USA, the prevalence of amoxicillin resistance has now reached 1% in a multicenter European study. There are indications that amoxicillin resistance is clinically relevant. The first cases of resistance against tetracyclin were reported in 1995 and 1996, and in 1997, a 6% prevalence of tetracyclin resistance was found in Italy.

Selection of second-line treatment should be based on individual assessment of post-treatment resistance, whenever feasible. In the case of ascertained combined resistance against nitroimidazoles and macrolides, the recommended second-line therapy is quadruple therapy, in which a PPI is added to the standard bismuth-based triple regimen. But even with this quadruple therapy, the *H. pylori* cure rate is reduced by metronidazole resistance. Therefore, in our clinical practice, a high-dose PPI-amoxicillin (e.g. omeprazole 40 mg t.i.d., amoxicillin 1,000 mg t.i.d. for 2 weeks) is the preferred second-line treatment in strains resistant to clarithromycin and metronidazole.

Future Aspects

Two questions for the future are whether a general cure from *H. pylori* of the whole population ('global eradication') is possible, and whether this would be desirable. A high prevalence of *H. pylori* infection in countries with limited medical resources and the emergence of microbial resistance will probably hinder global eradication with the currently used antibiotics. An antimicrobial agent targeted specifically at *H. pylori* is urgently needed. The development of such a drug may now be facilitated by the recent identification of the complete genomic sequence of *H. pylori*. This knowledge may also contribute to the development of vaccines. Vaccines have already been successfully tested in animal models, both with preventive and therapeutic intentions.

Whether the disappearance of *H. pylori* infection might be disadvantageous is currently the subject of lively discussion. The concept that *H. pylori* may be beneficial in some individuals for the prevention of gastroesophageal reflux disease is very controversial. Even if proved true, it would be outweighed by the benefits of curing *H. pylori* infection in most cases.

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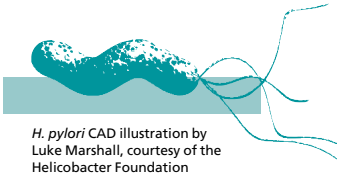

H. pylori CAD illustration by Luke Marshall, courtesy of the Helicobacter Foundation (www.helico.com)

Sleeping Sickness in Africa

On the Rise Again

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Fig 1. Tsetse fly (*Glossina* sp.)

African trypanosomiasis or sleeping sickness is an infectious disease caused by trypanosomes. These unicellular parasites are transmitted by the bite of infected tsetse flies (fig. 1) which act as vectors. The disease is restricted to Africa and to the distribution of the tsetse flies. Two species of trypanosome cause the disease, *Trypanosoma rhodesiense* (causing acute disease in East Africa) and *Trypanosoma gambiense* (causing chronic disease in West and Central Africa) [1]. Over 20 species of tsetse flies are known, but not all are equally important for transmission. Tsetse flies acquire the parasites while feeding on infected animals. In the flies' digestive tract, the parasites undergo a 3- to 4-week developmental cycle (fig. 2) before they reach the salivary gland and the fly becomes infective. The salivary gland infection rate is low, normally below 1%.

The pathogens can be harbored by both domestic and wild animals which then act as reservoirs for the disease. While domestic animals can show signs of disease, wild animals remain healthy (i.e. they are trypanotolerant). The livestock diseases Nagana and Surra are caused by other trypanosome species nonpathogenic for man. Animal trypanosomiasis causes huge economic losses and makes livestock production impossible in those areas where the infected flies are endemic. On the other hand, trypanosomes indirectly help in the protection of the environment and wild animals by keeping man and his domestic animals away from tsetse-infested regions including national parks.

The first symptoms of sleeping sickness appear at the site of the tsetse fly bite with a local reaction followed by unspecific signs such as fever, headache, muscle ache, and joint pain. During this first stage of the disease, which is sometimes mistaken for malaria, the parasites restrict themselves to the blood and lymph system (fig. 3), and only after weeks or months do they move into the CNS (second stage). Various neurological signs are characteristic of this stage: sleep disturbances, alteration of mental state, coordination disorders, and finally coma. If untreated, sleeping sickness ends fatally.

The parasites can be found in the blood, lymph node aspirate and cerebrospinal fluid. Because the trypanosome density is usually very low, detection of the parasites is difficult. Indirect methods (immunological, molecular) are available, but direct detection of the parasites is required before the stressful drug treatment is initiated. For this, concentration methods have to be employed, i.e. centrifugation or chromatography. Treatment is based on two drugs for the first stage and two drugs for the second stage (see below).

Epidemiology

Human pathogenic trypanosomes were only discovered at the beginning of the 20th century when the relation between sleeping sickness, tsetse flies and trypanosomes was first recognized. The disease in man and domestic animals had been known for much longer. On the basis of this new evidence, control strategies could be developed. Vector control can be done with traps or insecticides. There are various types of traps and they are all based on visual attraction, sometimes in combination with chemical attractants. Some species can be attracted by odors such as octenol or acetone, but not all. To make the trap, blue and black cloth is arranged as a screen or as a pyramidal construction. The blue color attracts the tsetse flies from a distance and they land on the black surfaces. The traps can work with chemicals which either kill or sterilize the insects or without anything; the tropical sun will finish the job, the flies die of desiccation.

Active surveillance and treatment of patients, which helps to reduce the human reservoir, is the second most important measure of controlling sleeping sickness. The animal reservoir is another aspect which needs to be considered. Especially domestic animals like pigs, cows and dogs can play a key role by harboring trypanosomes pathogenic to humans. Wild animals can also act as reservoirs. David Bruce was one of the first to realize this and he proposed, in 1913, to kill all the game animals in tsetse fly-infested areas by stating: 'It is self-evident that these wild animals should not be allowed to live in "fly-country", where they constitute a standing danger to the native inhabitants .... active measures should be taken for their early and complete blotting out'.

Other rigorous methods to reduce the tsetse fly population were used during colonial times: bush clearing which aimed at destroying the habitat of the vectors regardless of environmental damage, and the spraying of insecticides such as DDT.

Thanks to intense control measures organized by the colonial administrations, the disease had been brought under control by the 1960s (fig. 4). But since 1970, the situation has deteriorated: In many newly independent countries, political turmoil and economic crises have eroded the public health infrastructure necessary to monitor and control the disease. Today, actual prevalence levels are similar to those of the 1930s. The strong correlation between war and unrest and the number of sleeping sickness patients could be observed in the Congo, Angola, Sudan and Uganda. Before the civil war in Angola only a few cases of sleeping sickness occurred per year. Since 1975, when the civil war began, and with the collapse of sleeping sickness control measures, the disease has flared up. Today, some 100,000 or more patients are estimated to live in Angola. It is difficult to estimate the number of sleeping sickness patients worldwide today, but 300,000–500,000 seems to be a realistic figure. Over 30 countries in Africa have reported cases during the last 10 years, with the majority of patients living in the Congo, Angola, Sudan and north-west Uganda, all countries with war and unrest, and inadequate means of surveillance.

Treatment

The drugs used to treat sleeping sickness are rather old and rather toxic, and none can be taken orally [2]. For the first stage two drugs are available which do not cause major problems of resistance or toxicity. But for treatment of the second stage (2/3 of the patients seeking treatment),

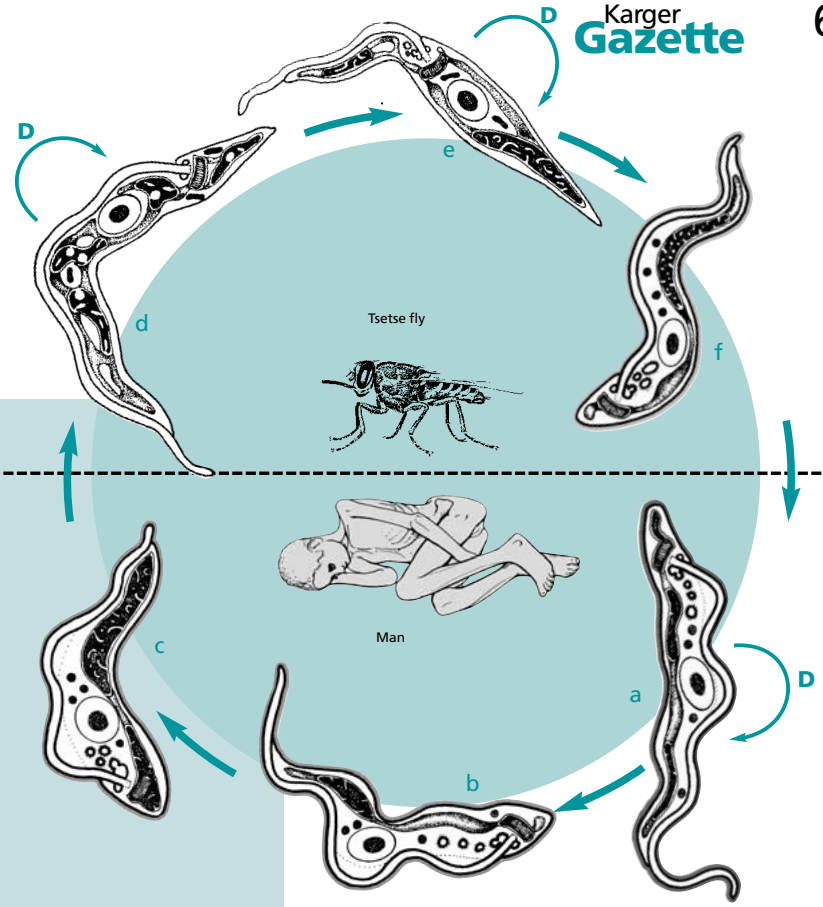

Fig. 2. Trypanosomes undergo a series of differentiations both in the mammalian host and in the tsetse fly. In the bloodstream of man, the parasites are covered by a dense coat of variable surface glycoproteins (VSG) and are infective for mammals. They exhibit a polymorphism with dividing (D) slender forms (a), intermediate forms (b) and stumpy forms (c). It is mainly the stumpy forms which, in the midgut of the tsetse fly, transform to midgut forms (d). These divide and further develop into the epimastigote forms (e). The midgut and epimastigote forms do not have a VSG coat. The epimastigote forms migrate to the salivary glands where they differentiate into the infective metacyclic forms (f), which, again, are covered by the VSG coat and are injected along with the insect's saliva into the next mammalian host. [Adapted from Vickerman K: Br Med Bull 1985;41:105–114.]

the situation is dramatic. Both drugs in use show problems of efficacy, availability, affordability, toxicity and resistance: the organo-arsenical drug melarsoprol (Arsobal®), which was developed over 50 years ago, is highly toxic and is not always effective; the second drug, eflornithine (Ornidyl®), which is used as an alternative or in cases of treatment failure, is not efficacious for one type of sleeping sickness, is enormously expensive and currently not available. Both drugs have to be administered intravenously over a period of 2–4 weeks (fig. 5). A new drug, therefore, is urgently needed, and it should be effective, nontoxic, cheap and available in tablet form. Currently, none of the pharmaceutical companies is developing new drugs for sleeping sickness or related diseases. The reasons for this are purely economic. The number of patients is limited and the people are poor – in other words: there is no market! The costs of developing a new drug are as high as USD 200–300 million, and companies are not prepared to invest such large amounts of money unless they can expect good returns within a reasonable period of time.

The void in the drug development sector caused by the pulling out of the pharmaceutical industry has been partly filled by a WHO programme and a European network of partnerships for drug discovery and development. The WHO/TDR programme 'Drug Development Research' is coordinating and funding drug discovery and preclinical research. Its aim is to identify new lead compounds and compile enough preclinical data to get the pharmaceutical industry interested in taking over the development of a new medication. The Swiss Tropical Institute (STI) is involved in these activities, especially in the evaluation of active substances in mouse models and a monkey model for sleeping sickness. Several studies have been carried out in collaboration with the Kenya Trypanosomiasis Research Institute (KETRI) outside Nairobi.

The Socioeconomic Burden

Sleeping sickness is a burden for communities because of its fatal outcome as well as its symptoms at the advanced stage. Neurological disorders can cause mental disturbance and changes in patient behavior which may be taken for madness. In this state, the patients are not aware of what they are doing and may even commit criminal acts which may result in their being made outcasts by the community. Due to the loss of work capacity, the consequences for an affected family are severe. It should also be kept in mind that a hospitalization of about 30 days imposes immense problems on the accompanying family members who have to look after the patients and prepare their food. This causes additional loss of work capacity. The economic consequences of the disease can be expressed in DALYs (disability of adjusted life years lost). Sleeping sickness is causing an annual loss of 1.8 million DALYs, which places this disease third among the parasitic diseases behind malaria and schistosomiasis:

The treatment costs for second-stage disease are high for melarsoprol and unaffordable for eflornithine (almost USD 1,000 per patient). Until now, WHO and nongovernmental organizations have been donating the drugs. Whether this policy can be maintained is questionable considering the increasing numbers of patients. Assuming treatment of 50,000 patients per year, the drugs required would cost USD 50 million.

STI and Sleeping Sickness Research

Trypanosomiasis research has deep roots at the STI. Professor Rudolf Geigy, the founder and first director of the institute, took a great interest in sleeping sickness and started studies in Tanzania in the late 1960s on the role of wild animals as reservoirs for human trypanosomiasis. In 1970 and 1971, I worked as a student in Uganda at the East African Trypanosomiasis Research Organization (EATRO), by that time the leading institution in sleeping sickness research; but this freshly established collaboration met with a sudden end after Idi Amin came into power and took the country into a civil war that lasted for 15 years. Since then, the STI has maintained an active research group working on tsetse flies and trypanosomes. Over the years, many scientists, technicians and students have contributed to the success and recognition of this group.

For the last 30 years, the sleeping sickness research of the STI has been focused on field studies in Tanzania and Uganda combined with laboratory research in Basel. This balance between field and lab research has been maintained throughout and still represents one of the strengths of the STI. Based on a growing expertise in methodologies for cultivating the parasites in vitro and using them for screening purposes, my group's interest moved towards chemotherapy. About 7 years ago, the group also embarked on pharmacological research on melarsoprol, which culminated in the proposition of a new treatment protocol for the drug [3]. Three years ago, a clinical study was started to validate this new treatment regime, with success as we know today. The future of the STI's sleeping sickness research will be an integrated approach to the problems of chemotherapy: from searching for new drugs to pharmacokinetic studies, and from laboratory studies on drug resistance to clinical studies with patients in Africa.

Research Projects in Africa

During the last 20 years, the STI was involved in collaborative research work in Kenya and Uganda. This research was supported by the Swiss Agency for Development and Cooperation, and by the WHO. Today, two main projects maintain this tradition: the IMPAMEL (improved application of melarsoprol) project in Angola, and an East-African network for sleeping sickness research and control.

The IMPAMEL project in Angola, managed by Dr. C. Burri, is validating a new treatment protocol for melarsoprol, based on pharmacokinetic investigations and computer simulations of his PhD thesis [3]. The new protocol is much shorter than the standard one (10 vs. 30 days) and uses 30% less drug. In a clinical study which involved 500 patients, IMPAMEL showed that the new treatment is as efficient as the old one with similar adverse effects. It will now be tested in several African countries and is likely to replace the old one soon.

In 1991, the STI revived its research partnership with the former EATRO in Uganda. The institute had changed its name to 'Livestock Health Research Institute' with a mandate for animal diseases and human trypanosomiasis. Collaborative research focused on the characterization of the trypanosomes causing the north-west Ugandan sleeping sickness epidemic, as well as on drug resistance monitoring. Training of students and staff was an integrated part of the collaboration as well as institutional support (training courses, technology transfer, donation of equipment and materials). Good links were also established with KETRI, the corresponding institute in Kenya, where pharmacokinetic studies on green monkeys were being carried out. Based on these two links and on links to equivalent institutes in Tanzania and Sudan, a network is currently being set up, aiming to promote research and control of sleeping sickness in the Lake Victoria region. The goal of this new network will be to strengthen collaborative research, technology transfer and communication among these neighboring countries. Also, training and capacity building are key issues of

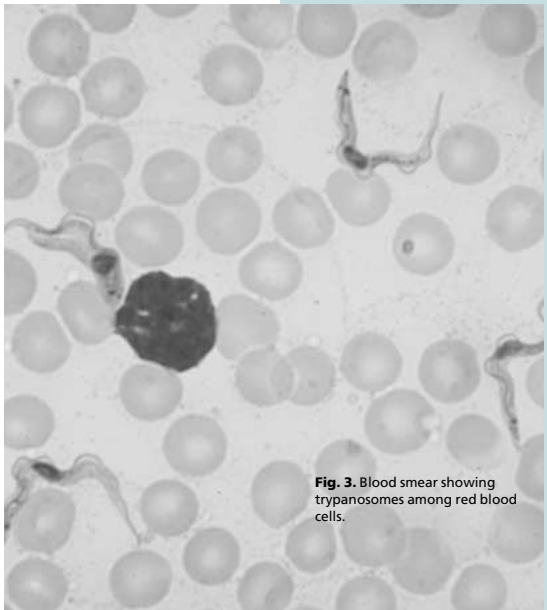

Fig. 3. Blood smear showing trypanosomes among red blood cells.

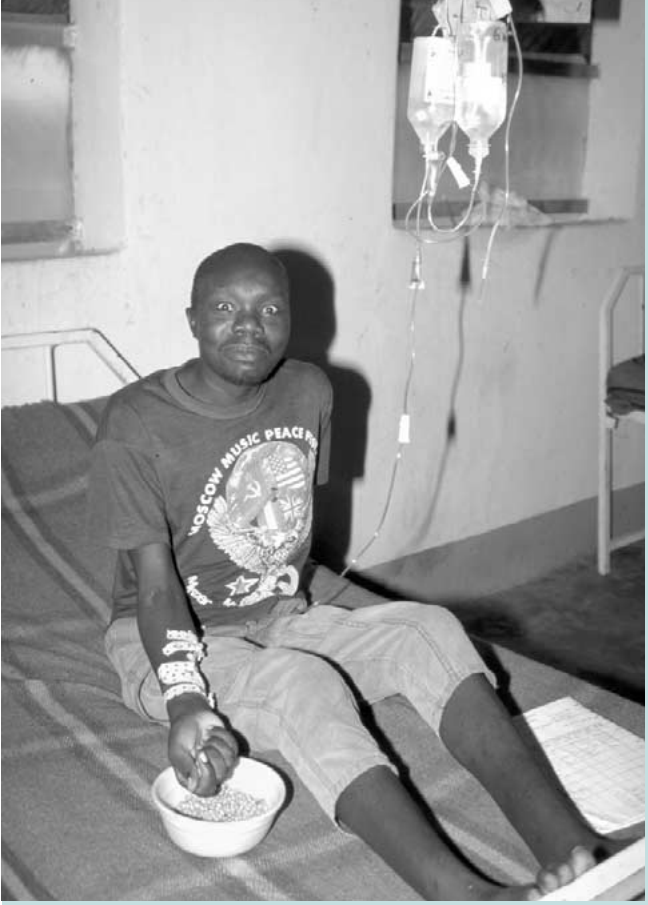

Fig. 5. Sleeping sickness patient in Omugo, Uganda. Typical are the 'moon face' (edema), the grasping reflex and the apathetic state.

this informal network, with the STI as a coordinator and research partner.

Main Problems for Researchers

The problems researchers working in East Africa are confronted with are manifold, but they can be grouped into logistic problems and open research questions. The logistic problems concern mostly our African research collaborators and include low salaries insufficient to support an average family; scarce funding for laboratory equipment, bad infrastructure and inadequate means of communication, which slow down productivity; and brain drain, i.e. an increasing number of young scientists are leaving the country to seek better living conditions. The HIV epidemic also adds to the difficult situation, a problem we have personally encountered by losing several of our local collaborators.

The major problems we are facing on the scientific level are: (1) resistance problems with the drug melarsoprol; (2) risk of spread of epidemic foci to other parts of the country, and (3) lack of reliable, quick and cheap methods to diagnose *T. gambiense* sleeping sickness. The first partial successes could be achieved during the past 7 years of collaboration, but more effort is needed to solve these problems.

Major Achievements of the STI

During the last 20 years of sleeping sickness research at the STI many research projects were carried out, some with minor and others with spectacular results; however, not all the results could be exploited directly for the benefit of the patients. The major achievements are:

- Discovery of genetic recombination among trypanosomes [4]
- Development of methods to grow trypanosomes in culture and assays to determine drug resistance and screen compounds for antitrypanosomal activity [5]
- Development of a new, improved treatment protocol for melarsoprol [3]
- Establishment of an informal East-African network for research and control of sleeping sickness.

The STI will continue its work on research problems in the area of sleeping sickness control in close collaboration with partners in Africa, the WHO and other researchers, committed to improving the desolate situation we are facing today. We will use our skills and knowledge to contribute to improve the treatment of sleeping sickness and thus reduce the number of people suffering and dying of this disease.

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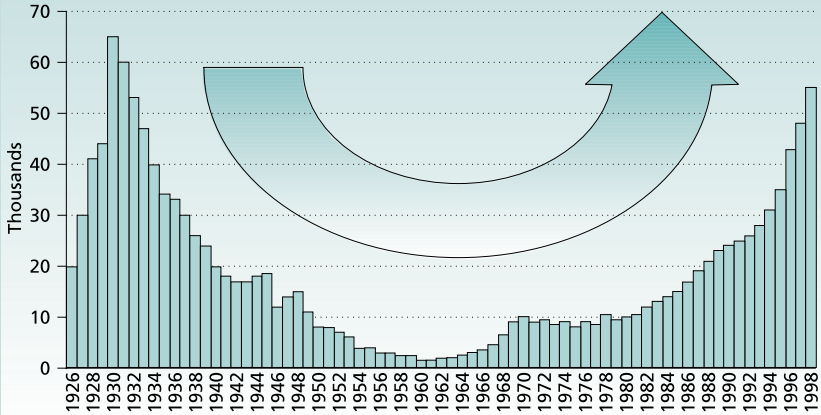
Fig. 4. Number of cases of sleeping sickness in Central Africa, 1926–1998 (source: WHO / CTD).



The Swiss Tropical Institute

The Swiss Tropical Institute (STI) was founded in 1943 by the late Prof. Rudolf Geigy. Since then it has developed into an institution for international health active in the areas of research, teaching and training, services and development cooperation. It works in close collaboration with the University of Basel, the Swiss Agency for Development and Cooperation (SDC), with WHO and other national and international organizations. Today, the STI employs approximately 140 people with an extended network of international collaboration.

There are four main areas of activity:

1. Research in medical parasitology and infection biology, epidemiology and public health. Research activities in the STI combine laboratory-based work with clinical and epidemiological studies. A major focus of research is malaria, especially molecular, genetic and immunological analysis of host-parasite interactions, immune reactions of the host, and potential new vaccines and drugs. A second focus is sleeping sickness, where research is aimed at the improvement of therapy through new treatment protocols with existing drugs and the discovery of new active compounds in close collaboration with WHO. A new area focuses on two other infections, meningococcal and respiratory infections. Epidemiological and public health studies deal with intervention studies for communicable disease control, health systems research and migration studies. These investigations are complemented by biometric and basic epidemiological studies. Malaria is also of focal interest, e.g. the control of malaria in endemic areas by insecticide-impregnated mosquito nets.

2. Teaching and training in undergraduate and graduate courses at the University of Basel and other Swiss universities and in STI's own courses. Courses of 1–12 weeks' duration are offered to a variety of health professionals who are working in developing countries or are preparing to do so. Scholarships offered by SDC and others have enabled students from developing countries to participate in these courses. Refresher courses for laboratory personnel and physicians are also offered.

3. Medical and diagnostic services. This department offers medical advice and vaccinations to people travelling to and returning from tropical countries (including a 24-hour emergency service), complete medical check-ups for patients as well as diagnostic services for physicians, hospitals and other laboratories.

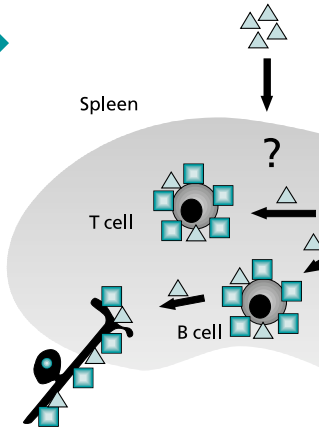
4. A support center for international health, which provides expertise and collaborates with governments and nongovernmental organizations in health planning, evaluation of health systems and health risk. A well-equipped library and documentation centre is open to the public. The STI also publishes the scientific journal *Tropical Medicine and International Health* in collaboration with other European Tropical Institutes.

For anyone interested in the current projects and activities of the STI, the institute's homepage offers detailed information: **http:/www.sti.unibas.ch**

Bruno Oesch, Markus Moser, Alex Raeber
Zürich

Prions

A Threat for the Next Century?



BSE (bovine spongiform encephalopathy; mad cow disease) belongs to a group of fatal, transmissible brain diseases called 'transmissible spongiform encephalopathies' (TSEs) or prion diseases. Prion diseases occur both in humans and animals and the denomination of the respective disease depends on the species in which it occurs (table 1). An infectious particle, the prion, is the causative agent of TSEs. In humans, several forms of Creutzfeldt-Jakob disease (CJD) have been linked to mutations in the prion protein gene (see below), resembling a classic genetic disease inherited in an autosomal-dominant fashion. Nevertheless, patients with Gerstmann-Sträussler-Scheinker syndrome (GSS), fatal familial insomnia (FFI) and familial Creutzfeldt-Jakob disease (FCJD) have been shown to produce infectivity by transmission to mice. It remains an enigma how a disease can be genetic and infectious at the same time.

The Pathogen

Prion diseases are caused by a novel type of pathogen, the 'prion', which differs from the known bacteria and viruses in many aspects: prions are extremely resistant to both heat and chemicals. Even prolonged heating to 100 °C (212 °F) and disinfection by most of the commonly used disinfectants does not lead to inactivation [1]. Prions are also hardly biodegradable [2]. Purification of the infectious particle has led to the identification of a protein (denominated prion protein) which is intimately associated with infectivity. Two forms of the prion protein exist: (1) a normal form (denominated PrP^{C}), expressed in animals and humans in a wide variety of tissues (brain, spleen, lymphocytes, lung, muscle), with unknown function, and (2) a disease-specific form (PrP^{Sc}) which has the same primary structure (i.e. amino acid sequence) but where the folding of the protein differs from PrP^{C}. Both forms of the prion protein are encoded by the same host gene [3]. Upon infection, the normal form of the prion protein is converted into PrP^{Sc}. In some human familial diseases, a mutation in the prion protein gene is tightly linked to disease, suggesting that the mutated prion protein might be spontaneously converted into PrP^{Sc}, thereby triggering off the disease process. No other component has been identified to be essential for infectivity. Prions, in contrast to viruses or bacteria, do not seem to contain pathogen-specific nucleic acids, which leads to the question: How do different strains of prions encode strain-specific inheritable information? While the presence of an undetected nucleic acid seems remote, the hypothesis that genetic information is encoded in the folding of the prion protein, though unprecedented, has gained acceptance due to the discovery of yeast proteins which have exactly this property. In yeast, [URE3] and [PSI] are nonchromosomal genetic elements which regulate nitrogen catabolism or transcription, respectively. The genetic information is encoded by the conformation of the proteins and this conformation is propagated to daughter cells. Despite the wealth of information indicating that the prion protein is a crucial part of the infectious particle, it has not been possible to formally prove that noninfectious normal PrP^{C} could be converted into PrP^{Sc} and thus become infectious. Until such proof is available, it is of paramount importance to remain open to other theories (i.e. unknown virus or bacteria), however unlikely they may seem at the moment.

Clinical Presentation

Prion diseases are characterized by a slowly progressive, invariably fatal degeneration of the central nervous system. Clinical symptoms include loss of movement coordination and, in humans, dementia; however, some variants of the disease like FFI have quite a different clinical presentation, i.e. patients suffer from sleep deprivation. Some of the patients may also be diagnosed as having Alzheimer's disease [4]. The interval between infection and occurrence of the first clinical symptoms is unusually long: 3–5 years in sheep, 5–7 years in cattle, over 10 years in humans, and 2 months to 1 year in experimental rodent models. In humans, the disease ends fatally often within a few months up to 2 years after the appearance of clinical symptoms. New variant CJD differs from classic CJD in that first mostly psychiatric symptoms (personality changes, depression, burning sensation) appear, followed only later by the classic symptoms of dementia or ataxia.

Transmission

The transmission routes of prion diseases have not yet been completely elucidated. The rapid spread of BSE in the UK and other countries seems to have been caused by feeding contaminated foodstuff to cattle. A second route of infection may involve maternal transmission to the embryo or the calf. In sheep, horizontal transmission also occurs via alternative routes which, however, are not fully characterized. Chronic wasting disease occurs in up to 5% of wild deer and elk, which suggests that there must be some natural transmission routes. The findings that prions are very stable in soil and that prions are secreted in feces, placenta or amniotic fluid by sheep point to the possibility of horizontal transmission.

The occurrence of a new variant form of CJD (vCJD) in the UK, France and Ireland and the scientific examination of the strain of prions in vCJD and BSE further suggest that BSE can be transmitted to humans while scrapie in sheep is generally considered nontransmissible to humans. Classic CJD has been transmitted through surgical procedures (cornea transplantation, dura mater grafting, use of stereotactic electrodes) or contaminated pharmaceutical preparations (e.g. growth hormone). More recently, the detection of prions in tonsils and other lymphatic tissues in vCJD patients has led to the suspicion that vCJD might be transmissible via blood products. Therefore, plasma and blood products from UK blood donors are no longer being used, and the FDA recommends deferring persons who have lived in the UK for a longer period of time (http://www.fda.gov/cber/gdlns/cjdnvcjd.pdf).

Table 1.
Transmissible spongiform encephalopathies /prion diseases

Creutzfeldt-Jakob disease (classical form)	CJD	humans
Creutzfeldt-Jakob disease (new variant form)	vCJD	humans
Gerstmann-Sträussler-Scheinker syndrome	GSS	humans [1]
Fatal familial insomnia	FFI	humans [1]
Familial CJD	FCJD	humans [1]
Bovine spongiform encephalopathy	BSE	cattle
Scrapie		sheep, goats
Transmissible mink encephalopathy	TME	mink
Chronic wasting disease	CWD	deer, elk
Experimental scrapie or BSE [2]		rodents, pigs

[1] Genetic forms of CJD associated with a mutation in the gene encoding for the prion protein. Inherited in an autosomal-dominant fashion.

[2] All the species with naturally occurring TSEs are also susceptible to experimental infection with prions. Rodents and pigs can be infected but (to date) have not been diagnosed with a naturally occurring prion disease.

Role of the Immune System

Following prion infection via peripheral routes, the prions are transported to the lymphoreticular system (LRS) where, during the preclinical phase of the disease, they replicate efficiently (fig. 1). It is noteworthy that the host fails to mount a classic immune response to prions, presumably because prions consist largely of a host-derived protein. In lymphoid organs, the earliest site of PrP^{Sc} accumulation and perhaps prion replication

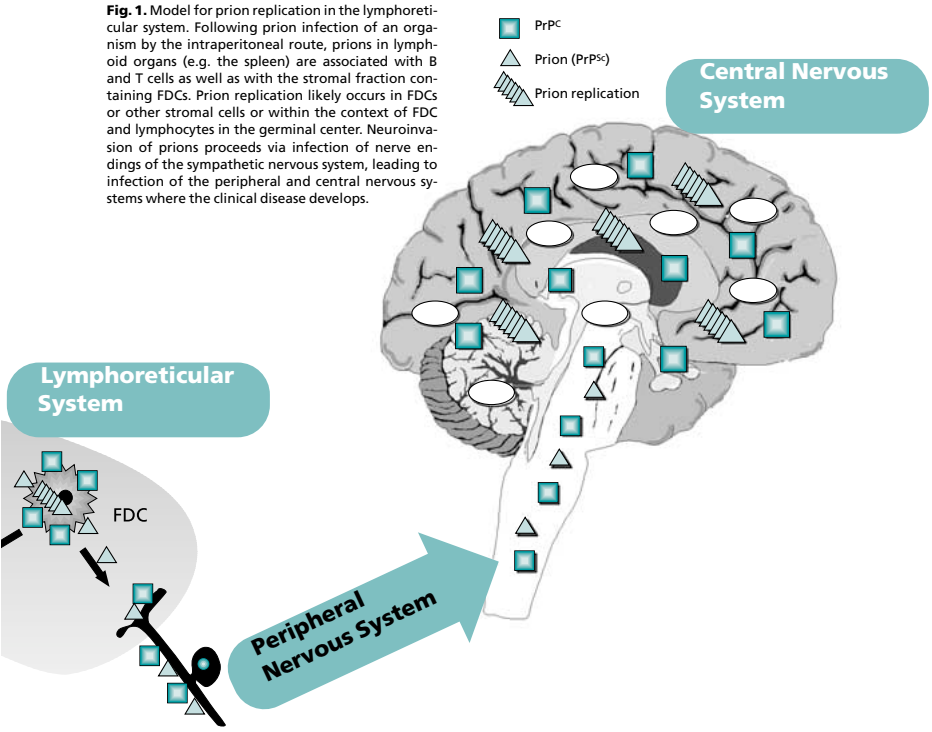


Fig. 1. Model for prion replication in the lymphoreticular system. Following prion infection of an organism by the intraperitoneal route, prions in lymphoid organs (e.g. the spleen) are associated with B and T cells as well as with the stromal fraction containing FDCs. Prion replication likely occurs in FDCs or other stromal cells or within the context of FDC and lymphocytes in the germinal center. Neuroinvasion of prions proceeds via infection of nerve endings of the sympathetic nervous system, leading to infection of the peripheral and central nervous systems where the clinical disease develops.

is the follicular dendritic cell (FDC), but how prions accumulate and might replicate within the context of the FDC network remains elusive.

Nevertheless, in the past 10 years we have witnessed an impressive leap in our understanding of the molecular mechanisms of peripheral prion pathogenesis. This progress has been achieved mainly by using transgenic and gene knockout technology. Experiments in transgenic mice with ectopic PrP expression indicate that neither B nor T cells alone are competent for prion replication [5]. Therefore, prions are synthesized either by FDCs or perhaps within the context of lymphocytes and FDCs. Although lymphocytes appear to be unable to replicate prions on their own, they are able to acquire prions in a PrP-dependent manner [6]. Since lymphocytes are in close contact with FDCs in the germinal center network, it is likely that the uptake of prions takes place through direct cellular interaction. It is attractive to speculate that prions might be localized to the surface of FDCs as antibody-PrP^{Sc} complexes in a similar manner as immune complexes are retained on FDCs for long-term maintenance of immune responses.

Following prion replication in the LRS, prions invade the CNS. Theoretically, there are two main possibilities: hematogenous spread or neural spread. The question whether lymphocytes play a role in the spread of prions from the periphery to the CNS is more than just academic. The extraordinary lymphotropism of vCJD prions has raised new public health concerns and demands urgent reassessment of the risk of vCJD being transmitted via blood or blood products derived from individuals suffering from preclinical prion disease. Although an intact immune system is required for prion neuroinvasion, several recent findings suggest that circulating prion-bearing lymphocytes – in particular B cells – might not be required to physically transport prions all the way to the brain. Firstly, prion-bearing lymphocytes were not detected in the blood of scrapie-infected mice, although splenic lymphocytes contained significant levels of prion infectivity [6]. More importantly, reconstitution of irradiated wild-type mice [7] or immunodeficient mice [8] with lymphohematopoietic stem cells devoid of PrP restored invasion of the CNS by prions following intraperitoneal inoculation. Collectively, these studies support the view that the hematogenous spread of prions does not play a major role in prion neuroinvasion.

Several lines of indirect evidence point to the peripheral nervous system as the crucial compartment that allows prions access to the central nervous system. Following prion infection via peripheral routes, prion replication in the peripheral nervous system always precedes the onset of replication in brain. The neuroinvasive process is thought to occur via infection of nerve endings of the sympathetic nervous system. Prions and PrP^{Sc} were found in the peripheral nervous system of scrapie-sick sheep, but so far PrP^{Sc} has not been detected in the autonomic peripheral nervous system. Interestingly, innervation of lymphoid tissue is, at least in part, controlled by lymphocytes themselves as both T and B cells secrete nerve growth factor, and, on the other hand, nerve terminals secrete a variety of molecules to stimulate the immune system.

For assessment of the risk of iatrogenic transmission as well as for the development of diagnostic and therapeutic strategies for prion diseases, a thorough understanding of the role of the immune system in peripheral prion pathogenesis is of immediate importance.

Public Health

To reduce the risk of infection for humans, veterinary officials in different countries have taken some or all of the following measures:

- Restricted use of meat and bone meal (MBM): The rapid spread of BSE seems to have been caused by feeding of foodstuff containing BSE-contaminated MBM. A ban on feeding ruminant-derived protein to ruminants was imposed in most countries in the EU as well as in the USA; however, a ban on feeding MBM to other species like pigs, fish and chicken has only been imposed in the UK.
- Ban on selling/using tissues with high levels of infectivity: brain, spinal cord, thymus, spleen and intestine, visible lymph and nervous tissue as well as lymph nodes. In the UK, beef on the bone containing ganglia close to the vertebral column is also banned.
- Ban on the use of animals with clinical symptoms: these animals are emergency slaughtered, rendered into MBM and burnt.
- Specifically in the UK, cattle over 30 months of age are not used for consumption. The question remains how many of these cattle still incubate BSE. In a survey, 18 of 4,951 cattle had indications of BSE [BSE Enforcement Bulletin No. 36, page 5, published by the British Ministry of Agriculture, Fisheries and Food].
- Increased surveillance: The Swiss Veterinary Authorities have started a surveillance program which is not only based on the observation of clinical animals, but targets specific populations like fallen stock and sick-slaughtered animals using a biochemical test for PrP^{Sc} (http://www.admin.ch/bvet/focus_bse/d/0_subfr.html).

Restriction of the use of MBM was probably the most effective measure which led to the decline of annual BSE cases in the UK and Switzerland (fig. 2). In other countries, especially Portugal, the number of cases is still on the increase. Since the ban on feeding MBM to ruminants was installed in the UK in 1988 and in Switzerland in 1990, most of the recent BSE cases were born after the ban, illustrating that either the measures taken against transmission of infectivity were not sufficient or that other modes of transmission might be possible: a future decline in BSE cases will indicate whether it has been brought under control or whether additional measures will be necessary. In this light, the development of vCJD cases in the UK is crucial. The 43 cases which have occurred to date might either be the persons particularly sensitive to infection with BSE or just the tip of the iceberg! Scientifically, it is not possible to predict which of these two scenarios will become reality. Decisions regarding additional measures might be necessary.

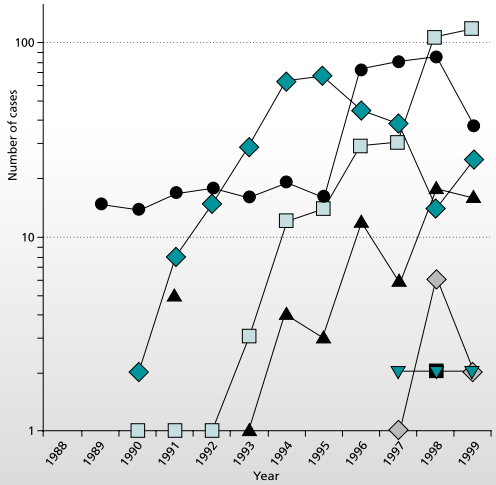


Fig. 2. BSE cases outside the UK (source: Office International des Epizooties, OIE).
1999: Numbers only up to middle of the year.

Summing up the Dilemma

BSE and vCJD have taken the public, politicians and scientists alike by surprise. While the public is insecure about the level of concern it should have regarding its food supply, politicians and scientists have been pushing the topic back and forth. Scientists will only know in 10 or more years whether BSE should be of major concern; however, when they do know, it will probably be too late to take preventive measures. In view of the dramatic potential of prion diseases, it might be appropriate to apply the strategy 'Better safe than sorry'. In some areas such as the blood product sector this strategy is already being applied, while in the food sector it is still hoped that BSE will disappear without any major measures being taken. Only the next century will reveal what would have been appropriate.

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Aspergillus and Aspergillosis

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Fig. 1. Growth of *A. fumigatus* on Sabouraud agar at 37 °C after 5 days of incubation (respiratory secretion of a patient with pulmonary aspergillosis).

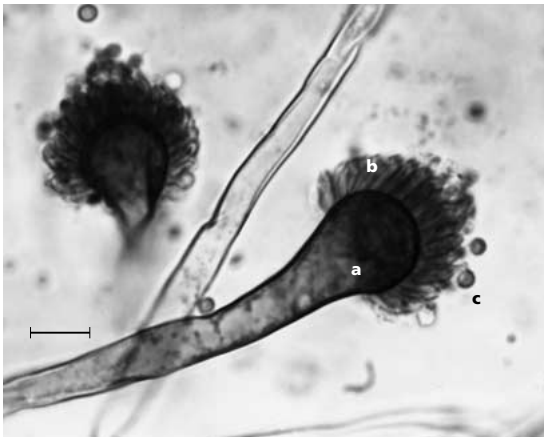
Aspergilli are moulds with a ubiquitous distribution in indoor and outdoor environments. Only some aspergilli can cause illness in humans, with *Aspergillus fumigatus* being the primary pathogen. The spectrum of diseases related to *A. fumigatus* is rather heterogenous, ranging from allergic disorders and localized infections such as sinusitis or bronchopulmonary aspergillosis to invasive, rapidly progressing and often fatal infections in the immunocompromised host. Animals, especially poultry, are also frequently affected by severe endemics of *Aspergillus* infections with an extremely high mortality rate.

In recent years, the prevalence of these opportunistic fungal infections has been increasing significantly, not least because of the growing number of immunocompromised patients worldwide. This development and its high mortality rate and social cost have made aspergillosis a major cause for concern. Elucidation of the virulence factors of the fungus which allow its installation within the host and the initiation of disease has become an important area of research over the past decade.

The Fungus

Under culture conditions (fig. 1), as well as on natural substrates such as decaying organic material, aspergilli exhibit septate mycelia and conidiophores. All *Aspergillus* conidiophores have a terminal vesicle. In the case of *A. fumigatus*, the vesicle only bears a single row of cells, termed phialides, which produce the conidia (fig. 2).

Fig. 2. Conidiophore with vesicle (a), phialides (b) and spores/conidia (c) of *A. fumigatus*. Bar =10 µm.



The production of conidia represents the asexual, anamorphic form of reproduction. Some *Aspergillus* genera and/or species are also able to reproduce in a sexual, teleomorphic phase. The genus *Aspergillus* is divided into about 15 different groups containing over 150 species. Most of the aspergilli show their growth and sporulation optimum at higher temperatures – mostly above 20 °C – which explains their higher prevalence in subtropical and tropical outdoor environments than in colder outdoor environments. In indoor environments, high concentrations of *Aspergillus* spores/conidia can be found in the summer, during heating seasons in winter, and often in the case of indoor building activities. As many aspergilli are thermophilic fungi, they can be found on decaying material, and humans are constantly being exposed to *Aspergillus* spores/conidia by inhalation of aeroplankton.

Aspergilli as Human Pathogens

In humans, almost all the invasive *Aspergillus* infections are caused by *A. fumigatus*. This species was first described by Fresenius in 1863 who isolated it from an airway infection in poultry. *A. fumigatus* shows high morphological and antigenetic variability, particularly in isolates from clinical specimens. In invasive human infections, *A. flavus*, *A. glaucus*, *A. niger*, *A. restrictus*, *A. terreus* and *A. versicolor* have also been described as causative agents, although they are rare and of minor medical importance.

Clinical Manifestations

Allergic Aspergillosis. Allergic aspergillosis can be caused by the inhalation of *Aspergillus* spores/conidia or different airborne metabolites of aspergilli. Acute bronchopulmonary aspergillosis (fig. 3) is increasingly being observed. Peribronchial eosinophilic infiltrations may occur and the disease can lead to exogenic allergic alveolitis, also known as 'farmer's lung'. Apart from that, patients can develop a status asthmaticus as a further complication. Acute allergic conjunctivitis and/or rhinitis have also been reported. Most of the allergic diseases caused by *Aspergillus* species are attributable to *A. fumigatus*.

Invasive Aspergillosis. *A. fumigatus* is the most frequent species isolated in invasive aspergillosis, followed by *A. flavus*. Invasive aspergillosis is nearly always an opportunistic infection and occurs especially often in the granulocytopenic host. The route of infection is mostly by inhalation of *Aspergillus* spores/conidia. The incidence of invasive aspergillosis has increased significantly during the last decades, and this seems to be due to more aggressive therapy regimens, especially in antineoplastic therapy and in transplantation medicine. Hematogenous dissemination can occur into almost all organs (fig. 4, 5). Further, a tendency to peripheral thromboembolism and bleeding disorders such as hemoptysis (coughing up of blood) can be seen. The definite diagnosis of invasive aspergillosis is often only confirmed postmortem, as diagnostic options – especially in the immunocompromised host – are inadequate. Invasive aspergillosis is a life-threatening infection with an extremely high mortality rate even after appropriate antimycotic treatment. Hygiene plays an important role in avoiding and minimizing the exposure to *Aspergillus* spores/conidia.

Localized Aspergillosis. Aspergillomata are fungal balls consisting of compact fungal mycelia which can be found as a localized infection/colonization in preformed cavities such as old tuberculous cavernae, or in bronchiectasis (fig. 6, 7). Hemoptysis occurs frequently. The tendency to gen-

eralization is almost negligible and, again, *A. fumigatus* is the most frequently found species in this disease. Otomycoses of the outer ear channel – mostly due to *A. niger* – and *Aspergillus*-associated infection of the paranasal sinuses are further localized aspergilloses.

Mycotoxicosis. Plenty of mycotoxins such as aflatoxins, ochratoxin and patulin are produced by different *Aspergillus* species and/or isolates. These mycotoxins can be ingested with food, e.g. grains or contaminated dairy products, or inhaled. The toxicity of aflatoxins, mostly produced by strains of *A. flavus*, has been well studied: They show hepatotoxic, hepatocarcinogenic, mutagenic and teratogenic effects in animals. In humans, aflatoxins are hepatotoxic and seem to be a causative agent for Reye's syndrome. The amount of mycotoxins contained in food is mainly dependent on storage conditions: aeration should be ensured by taking care that foodstuffs are not packed too tightly. This is especially dangerous in warm, humid conditions and climates.

Diagnosis

Definite diagnosis of allergic aspergilloses is often obtained by endobronchial exposure to the allergens although this procedure is risky, especially because of the possibility of life-threatening allergic reactions. Further, serum antibody tests and the radioallergosorbent test (RAST) give important information.

Invasive infections can be detected by imaging procedures such as X-ray, CT scan and sonography, but laboratory confirmation is of great importance. Respiratory secretions, bronchoalveolar lavage fluid as well as other clinical specimens from infected sites should be examined both under the microscope and in cultures. There have been a lot of cases where the culture results were negative despite the presence of mycelia typical of *Aspergillus* being found microscopically, e.g. in smears or histopathological sections, which is a frequent occurrence when specimens are obtained after the onset of antimycotic therapy. Many isolates from clinical specimens show an atypical morphology. Positive cultures must be confirmed, as concomitant aeroplankton contaminations can lead to false-positive results. Some problems can arise with serological procedures in the diagnosis of aspergillosis. Patients with allergic aspergilloses often show high specific IgG antibody titers against *Aspergillus* polysaccharide and/or glycoprotein antigens combined with high serum IgE titers and also often specific anti-*Aspergillus* IgA titers. On average, about 90% of the patients with aspergillomata show specific IgG antibody titers against *Aspergillus* antigens, often combined with specific IgA titers. In contrast, only 70% of the patients with allergic aspergillosis and less than 30% of the patients with severe invasive aspergillosis show specific IgG antibody titers.

Aspergillus antigen detection is another option. This can be performed by different methods such as RIA, ELISA and latex agglutination test on serum probes or urine. The main limiting factor of specific antigen detection – mostly consisting of galactomannans – is their short half-life in clinical specimens, thus making it necessary to take multiple probes if valid results are to be achieved. Altogether, the interpretation of serological results in *Aspergillus*-associated diseases is problematic and must aways be combined with clinical diagnostic procedures, and microscopic and microbiological techniques.

Therapy

Allergic diseases are generally treated symptomatically, and it is important that contact with the causative allergen be eliminated as far as possible. Exposure to aeroplankton containing *Aspergillus* spores/conidia or metabolism products can be significantly reduced or influenced by altering the indoor environment (e.g. by constantly ventilating rooms, decreasing the air humidity, not placing furniture too close to the walls, reducing the number of potted plants).

Systemic aspergilloses are life-threatening opportunistic infections which require a specific antimycotic therapy. In some cases, antimycotic therapy is able to stop the infection but has no curative effect until the patient's underlying state of immunosuppression has improved. Also, prophylactic intranasal application of amphotericin B has been reported to be useful in patients at risk, as invasive aspergilloses can sometimes arise from a localized *Aspergillus* sinusitis, especially in immunocompromised patients.

Despite its high nephrotoxicity, amphotericin B is the first-line drug for systemic therapy. There is an urgent medical need for more efficient and less toxic antimycotic compounds since the frequency of invasive aspergillosis is steadily increasing due to the 'progress' of modern medicine which has resulted in a higher number of patients being severely immunocompromised as a consequence of anticancer chemotherapy or therapy with immunosuppressive drugs, corticosteroids or broad-spectrum antibiotics. Newly developed azole antimycotics have shown promising first results in clinical trials.

In vitro sensitivity tests can be performed by different dilution and diffusion techniques. However, no standardized technique is yet available (e.g. NCCLS standard). Furthermore, there is an extremely poor correlation between in vitro sensitivity data and the clinical outcome of therapy. We observed [unpubl. data] that there is also an extremely poor or no correlation between sensitivity data and the therapeutic efficacy of the drug in murine experimental generalized aspergillosis. This led us to suggest that in vitro sensitivity tests of *Aspergillus* strains are pointless for selecting antimycotic therapy regimens.

In the case of aspergillomata, a cavernoscopic elimination of the fungal material with consecutive local instillation of amphotericin B is the therapy of choice. Resection of aspergillomata can be critical as this disease often causes severe bleeding with massive coagulation problems during surgery.

The following section deals with the question of what makes *Aspergillus* such an aggressive pathogen. Though mainly aimed at scientists, this discussion may also be valuable for doctors interested in the molecular pathogenesis of aspergillosis.

Virulence Factors of *A. fumigatus*

The best test for identifying a virulence factor is comparison of the infectivity of the fungus in the absence and in the presence of the putative factor. Using this method, putative virulence factors

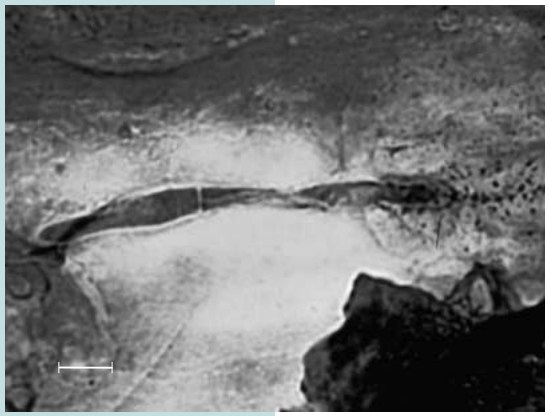


Fig. 3. Septate mycelium of *A. fumigatus* (culturally confirmed) in a respiratory secretion of a patient with acute bronchopulmonary aspergillosis. Gram staining. Bar = 10 µm.

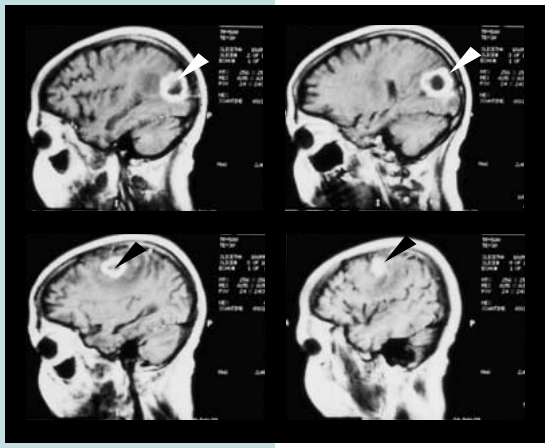


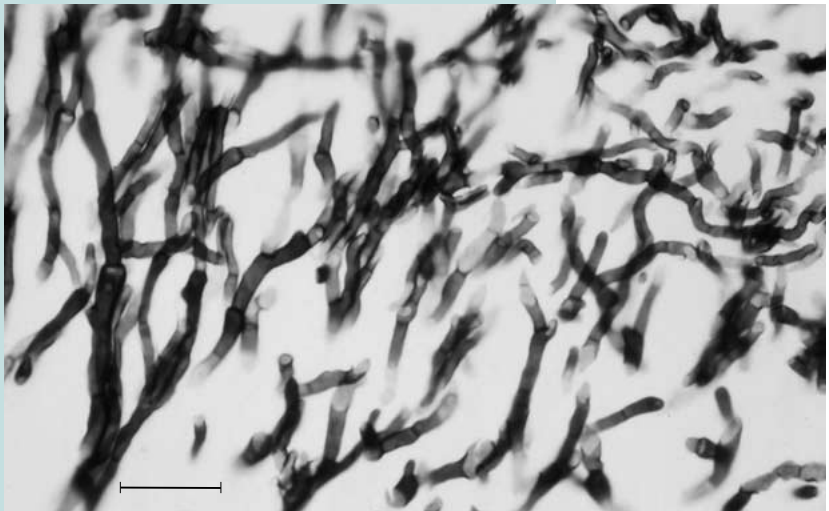
Fig. 4. Cranial CCT with a parietal and occipital brain abscess caused by *A. fumigatus* in a 14-year-old patient with acute myeloid leukemia.

such as proteases and a toxin (ribotoxin) have been analyzed. However, with the exception of 1,8-dihydroxynaphthalene (DHN)-melanin (see below), none of the factors analyzed until now managed to reduce virulence in a murine infection model when the corresponding gene was deleted.

The importance of DHN-melanin for virulence was elucidated based on the following rationale: For the disease to develop, conidia have to survive in the host and germination has to start. This indicates that some factors attributed to the conidia of *A. fumigatus* help the fungus survive the attack by the host's residual immune system. Because of their abundance in the air, *A. fumigatus* conidia are continuously inhaled during routine daily activities. Mucociliary clearance and phagocytic defense normally prevent the disease, but a severely depressed immune system provides an opportunity for conidia to germinate and invade lung tissue.

We reasoned that one of the factors which contribute to conidial survival might be their gray-green pigmentation. This reasoning is also based on observations with other fungi, e.g. *Cryptococcus neoformans*, a fungus with a pathogenic yeast phase, which causes life-threatening infections, particularly in AIDS patients. It has been shown that production of a melanin-like pigment is a virulence-determining factor in *C. neoformans*. One mechanism by which pigment might contribute to virulence derives from its ability to confer some resistance to reactive oxygen species (ROS), a major host antimicrobial effector system. Therefore, a mutant strain of *A. fumigatus* was isolated which had lost the ability to produce the gray-green conidial pigment. Conidia of this mutant are

Fig. 5. Histological section from the brain abscess shown in figure 4. Grocott silver staining. Bar = 20 µm.



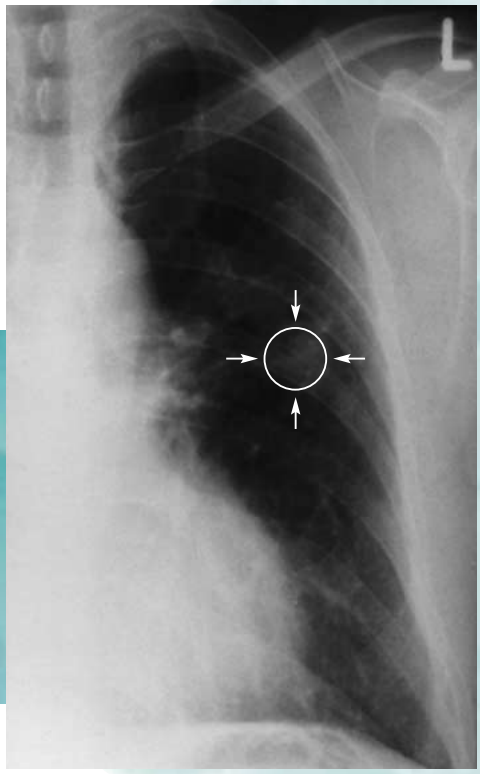


Fig. 6. Aspergilloma (see arrow) caused by *A. fumigatus* in a 43-year-old patient. Chest X-ray.

white ('W') and, in addition, have an altered surface. The surface of wild-type ('WT') conidia is rough due to ornamentation. This surface structure is lacking on 'W' conidia, which have a smooth surface. In an intravenous murine infection model, the virulence of 'W' conidia was significantly reduced compared with that of 'WT'conidia. A gene was cloned which complemented all phenotypes of the 'W' mutant. The gene was designated *pksP* for 'polyketide synthase involved in pigmentation', and is 6,660 bp in size. 'W' mutant strains complemented to the gray-green phenotype of 'WT' conidia using the polyketide synthase encoding *pksP* gene showed a complementation of all other phenotypes including virulence in a murine infection model.

The *pksP* gene is part of a cluster. Upstream of *pksP* is the *arp1* gene; its product is very similar to scytalone dehydratase, an enzyme involved in DHN-melanin synthesis in *Magnaporthe grisea*, a fungus pathogenic in plants. Downstream of *pksP* is a gene with high similarity to 1,3,6,8-tetrahydroxy-napthalene (THN) reductase genes. It was therefore designated *thnr*. Based on previous results on DHN-melanin, it is very likely that the *thnr* gene product is also involved in the DHN-melanin pathway.

The fungicide tricyclazole specifically inhibits the reductases involved in DHN-melanin biosynthesis in black fungi. Inhibition of conidial pigmentation of *A. fumigatus* by tricyclazole suggests that the gray-green pigmentation of *A. fumigatus* was synthesized via a similar pathway to that of the DHN-melanin pathway in *M. grisea*. This was further supported by the identification of the gene cluster of *A. fumigatus* mentioned above. Interestingly, in contrast to *A. fumigatus*, the *Emericella nidulans* conidial pigmentation was not inhibited by tricyclazole. Taken together with other observations, this suggests that dissimilar pathways exist in *A. fumigatus* and *E. nidulans* pigment synthesis. Therefore, the DHN-melanin-containing conidial pigment seems to represent a characteristic feature of *A. fumigatus* which is a prerequisite for its virulence. It will now be of considerable interest to analyze the differences in the types of melanin and the regulation of their biosyntheses between the pathogenic *A. fumigatus* and the nonpathogenic *E. nidulans.* This can be expected to have a major impact on the understanding of the molecular basis of *A. fumigatus* virulence.

The mechanism by which the pigment might protect the conidia against attack by immune effector cells has not yet been clarified. 'W' conidia are 10 times more sensitive to ROS. They also induce a 10 times higher ROS release when coincubated with human polymorphonuclear leukocytes or monocytes. 'W' conidia exhibited significantly elevated complement component C3 binding capacity compared with 'WT' conidia. Inefficient deposition of complement component C3 on 'WT' *A. fumigatus* conidia has previously been demonstrated.

There are three possible explanations: First, the pigment scavenges ROS and helps to protect conidia from the attack by ROS produced by immune effector cells. Second, 'W' conidia were better recognized by phagocytes because the conidial surface of the 'W' mutant markedly differed from that of 'WT' conidia. This might result in a more efficient and stronger activation of phagocytes, which would explain the observed increase in production of ROS when the immune cells were challenged with 'W' mutant conidia. Consequently, the pigmentation helps 'WT' conidia hide from the immune system. Third, it has been known for a long time that *A. fumigatus* produces immunosuppressive substances. The nature of these compounds has not yet been elucidated. It is thus conceivable that intermediates of the pigment synthesis or the pigment itself have immunosuppressive activity.

Fig. 7. Aspergilloma (fungus ball) caused by *A. fumigatus* in a lung lobe after tuberculosis. Section material.

Outlook

Aspergillosis is one of the most feared infectious complications in leukopenic or transplant patients, and during the last two decades improvement in the prognosis for invasive aspergillosis has not been sufficient. It is hoped that the new molecular approaches in *Aspergillus* research will help to further elucidate *A. fumigatus* pathogenicity and ultimately lead to more effective therapeutic regimens, not just for humans but also for improved animal health. Prevention of hospital-acquired infections is of utmost importance, and more epidemiological research is needed to reduce the risk of exposure to infectious conidia by identifying and eliminating the potential sources of infection.

Suggested Reading

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