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Influence of microbial infections on the progression of HIV disease

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Over 10 years ago it was discovered that HIV is the etiological agent of AIDS. Cohort studies of HIV-seropositive subjects, particularly in the USA, showed that disease progression is highly variable among individuals. Several factors are thought to be responsible for this variation (Box 1). The genetic diversity of HIV itself has been recognized as one of these factors; for example, HIV-2-infected individuals progress towards disease more slowly than HIV-1-infected individuals². Moreover, in some long-term non-progressors (LTNPs), HIV-1 has been shown to bear a deletion in the *nef* gene, which probably reduces its virulence³; however, this deletion is not found in all LTNPs (Refs 16,17). Other factors are host-associated. Some are genetic; in particular, the expression of some human leucocyte antigen (HLA) class I or class II alleles has been associated with resistance or susceptibility to disease progression. Moreover, genetic studies in subjects who remain uninfected, despite being repeatedly ex-

HIV infection is associated with immune activation, which in turn stimulates HIV replication. Certain other co-infections cause immune activation and may contribute to an increased viral load.

The impact of co-infections by microorganisms such as *Mycobacterium tuberculosis* can be important for patient survival, particularly those at high risk of exposure to infection and with poor access to medical care.

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posed to HIV, reveal that resistance is related to a homozygous deletion within the *CCR-5* gene, which encodes the principal chemokine receptor for macrophage inflammatory protein 1 α (MIP-1 α) and 1 β (MIP-1 β) and RANTES (regulated upon activation, normal T-expressed and secreted factor) and serves as a co-receptor on macrophages for certain strains of HIV-1 (Refs 6,7). Interestingly, patients that are heterozygous for this deletion are not protected from infection by HIV-1 but seem to progress more slowly to AIDS than patients homozygous for wild-type *CCR-5* (Refs 18,19). Age is also an important predictive factor and is independent of genetic factors. Among adults, it is estimated that the risk of progression increases by 40% for each decade of life at the time of infection^{8,20}. Moreover, certain life history factors (including alcoholism, smoking, pregnancy, multiple exposures to HIV and continued intravenous drug abuse) have been suggested to increase disease progression, although there is no definitive evidence of an association⁹.

Another controversial factor is related to the mode of transmission of HIV. Results obtained with the French SEROCO cohort, a cohort of 1453 HIV-infected homosexual men and heterosexuals of both sexes, indicate that progression to AIDS is faster among male homosexuals than among haemophiliacs or intravenous drug users [with a relative adjusted risk of 1.8 (1.1–3.5)]^{10,20}. Several hypotheses have been proposed to explain these observations, including the earlier spreading of the epidemic among homosexuals, which could contribute to the selection of HIV strains characterized by increased virulence and/or lower sensitivity to viral inhibitors, as well as psycho-sociological factors, microbial co-infections (particularly those that are sexually transmitted) and even a bias in the recruitment of patients.

In addition to HIV and host-associated factors, infectious cofactors have been implicated both in the acceleration of the development of AIDS from the clinically asymptomatic phase and in the shortening of the AIDS phase itself. These cofactors include viruses, such as cytomegalovirus (CMV), human herpesvirus types 6 and 8 (HHV-6, HHV-8), herpes simplex virus type 1 (HSV-1), Epstein–Barr virus (EBV) and human T-cell leukaemia virus types 1 and 2 (HTLV-1, HTLV-2) (for review, see Ref. 11), several parasites, including *Leishmania donovani* and *Toxoplasma gondii*, and various bacteria. Although it would be expected that any co-infection would result in an additional assault to the immune system and, consequently, a faster progression towards AIDS, epidemiological studies have failed to demonstrate such an effect after *Plasmodium falciparum*²¹ infection or hepatitis C co-infection²². The apparent absence of an interaction between *P. falciparum* and HIV infection is remarkable because similar alterations of the immune system have been reported in both infections, including an increased lymphocyte apoptosis *ex vivo*²³ and Th1 vs. Th2 cytokine pattern evolution. However, a possible influence of malaria on HIV disease progression cannot be excluded because of the limited number of epidemiological studies performed^{21,24}. It is also noteworthy that although many microorganisms can potentially act as HIV cofactors, their influence on the progression to AIDS appears less significant than expected²⁵.

As there are marked differences in the geographical prevalence of infectious agents, differences are expected in the nature of opportunistic infections in HIV patients, as well as in the magnitude of their immune deficiency¹². Indeed, the major pathologies of African AIDS patients are distinct from those of patients living in industrialized countries. For example, tuberculosis (TB) and other bacterial pneumonias are more prevalent in Africa, whereas CMV infections, *Pneumocystis carinii* pneumonias (PCP) and atypical mycobacteriosis are more common in industrialized countries²⁶. However, as these infections disseminate in patients with low CD4 T-cell counts, it is possible that only a few African patients survive long enough to develop these opportunistic infections²⁶. There is strong evidence that these dissimilar patterns of disease are related to greater exposure to infectious agents in the African environment and to the lack of access to medical care. Indeed, a re-

Box 1. Cofactors in HIV infection

Viral factors

- HIV genetic diversity¹
- HIV-1 vs. HIV-2 (Ref. 2)^a
- *nef* deletion in long-term non-progressors^{3,a}
- Drug resistance^{4,a}

Host factors

- Human leucocyte antigen (HLA) class I and class II alleles^{5,b}
- Chemokine receptor (CCR-5) deletion^{6,7,c}
- Age^{8,a}
- Life history factors^{9,b}
- Mode of HIV transmission^{10,b}

Infectious factors

- Viruses (cytomegalovirus, human herpesviruses, Epstein–Barr virus, etc.)^{11,b}
- Parasites (*Leishmania donovani*, *Toxoplasma gondii*, *Pneumocystis pneumoniae*, etc.)^{12,b}
- Bacteria (mycobacteria^a, mycoplasmas^b, etc.)^{13–15}

^aConfirmed, large effect on HIV disease progression.

^bControversial effect on HIV disease progression.

^cSlight effect on HIV disease progression.

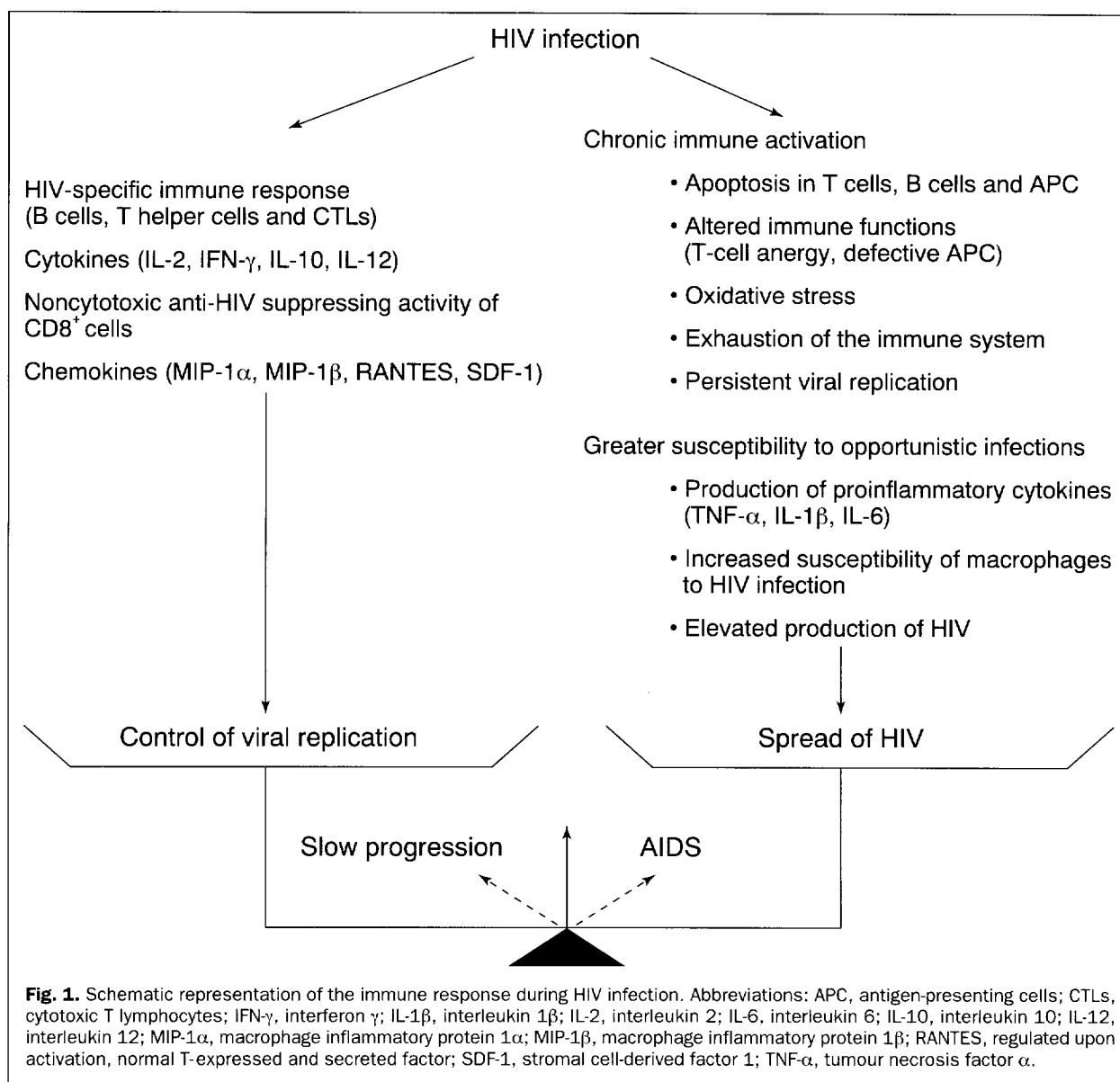
cent study showed that the mean CD4 T-cell level of HIV-infected individuals with AIDS-defining opportunistic infections is similar among Africans living in the UK and Europeans living under the same conditions²⁷. In this review, we will focus mainly on the causes and consequences of microbial infections that may accelerate the progression of HIV infection.

HIV infection and chronic immune activation

HIV-associated immune activation and consequences for immune function

A general state of immune activation develops rapidly during the early asymptomatic phase of HIV infection. This is reflected by follicular hyperplasia and extension of the follicular dendritic cell (FDC) network²⁸ in the lymph nodes, increased production of various activation markers, including β 2-microglobulin, neopterin, soluble interleukin 2 receptor (IL-2R), soluble CD8 molecules and soluble tumour necrosis factor α (TNF- α) receptors, and the expression of activation molecules, such as HLA-DR, CD38, CD57 and CD45RO (Refs 29–31) on lymphocytes, particularly on CD8⁺ T cells. Although resting lymphocytes can be infected by HIV, they become more sensitive to this retroviral infection once activated. Cell activation is also necessary for initiating HIV replication in cells containing the latent provirus. The relationship between the level of immune activation and the active replication of HIV dictates the plasma viral load (Fig. 1), which represents the outcome of viral production and the eradication of viral particles and infected cells by the immune system. It is considered to be the best predictive marker for monitoring disease progression³².

Various factors are thought to be involved in the general state of immune activation during HIV infection, including viral proteins, the cytokine imbalance and the chronic specific activation of effector T cells by the virus itself or by superantigens (SAGs). SAGs are proteins



involved in the pathogenicity of different bacterial (including *Staphylococcus aureus*, *Streptococcus* spp. and *Mycoplasma arthritidis*) and viral (including rabies virus, EBV, CMV and murine mammary tumour viruses) infections³³. These molecules stimulate an important fraction of T cells by binding to major histocompatibility complex (MHC) class II molecules of the SAg-presenting cell, as well as to V β elements of the T-cell receptor (TCR). A role for SAg in HIV infection has been proposed because of similarities with murine retrovirus infections, which are known to express SAg (for review, see Ref. 34). Although several HIV products (gp120, gp160 and Nef) are candidates for superantigenic activity, it is possible that SAg from other infectious agents could be implicated^{35,36}. In parallel to cellular activation, T-cell unresponsiveness (anergy) occurs before the number of CD4 T cells declines. Functional defects of helper T cells are observed in patients' lymphocytes, as characterized by the impairment of TCR-dependent activation *in vitro* in response to MHC-

restricted recall antigens or anti-CD3 monoclonal antibodies³⁷. Interestingly, the anergic state can be reproduced *in vitro* following incubation of normal CD4 T cells with the HIV envelope protein gp120 (Ref. 38). Moreover, it has recently been observed that the V γ 9V δ 2 subset, known to be triggered by *Mycobacterium tuberculosis* antigens, is anergic in some patients during primary HIV infection³⁹. The patients' V δ 2 T cells were unresponsive *in vitro* to mycobacterial lysates and to the natural nonpeptidic phosphoantigen ligand specific to V δ 2 T cells purified from *M. tuberculosis*^{39,40}. Although the precise role of $\gamma\delta$ T cells in anti-TB immunity is not known precisely, dysfunction of this subset may contribute to the development of TB in AIDS patients.

Another consequence of HIV-driven immune hyperactivation is the induction of programmed cell death (apoptosis) in uninfected T cells from HIV patients (for review, see Ref. 41). Indeed, all blood mononuclear cell subsets have been shown to be inappropriately programmed for apoptosis in HIV patients, and the onset of

apoptosis is significantly correlated with the activation state of the immune system. HLA-DR⁺ and CD45RO⁺ T cells, in particular, are more prone to apoptosis *in vitro* than their counterparts from uninfected individuals⁴². The persistent stimulation of the immune system is responsible for the dysregulation of the expression of molecules involved in cell survival (Bcl-2) or cell death (Fas) and induces an increased susceptibility to Fas-triggered apoptosis in lymphocytes⁴³. Exacerbated programmed cell death has also been detected *in vivo* in patients' lymph nodes⁴⁴, and it is noteworthy that the lack of immune activation observed in HIV-infected chimpanzees that are resistant to AIDS is also associated with a lack of inappropriate lymphocyte apoptosis⁴⁵. One of the consequences of increased apoptosis in AIDS is the premature disappearance of activated but functional cytotoxic and helper effectors.

Chronic oxidative stress is another characteristic of HIV infection. It is manifested, in particular, by a reduced concentration of the intracellular anti-oxidant glutathione (for review, see Ref. 46). One of the proposed mechanisms to explain oxidative stress involves the retroviral Tat protein, which is capable of inhibiting the production of superoxide dismutase (Mn-SOD), a key enzyme in the regulation of cellular redox⁴⁷, in human cells *in vitro*. The inhibitory effects of the Tat protein on human lymphocytes is not observed on the lymphocytes of chimpanzees⁴⁸. Opportunistic infections can also increase the oxidative stress induced by HIV (Ref. 49), thereby increasing HIV replication and the sensitivity of cells to cytotoxic agents, in particular TNF- α . It might also be at least partly responsible for the elevated apoptosis of patients' lymphocytes, as oxidative stress is an early inducer of the apoptosis process⁵⁰.

Influence of antigenic stimulation on HIV replication in vivo

Early studies suggested that specific or nonspecific activation of the immune system of HIV-infected individuals could increase the plasma viral load, at least temporarily. The most convincing data has emerged from studies analysing the influence of vaccination on the plasma viral load of HIV patients. Previous studies in animal models [HIV-1-infected chimpanzees and simian immunodeficiency virus (SIV)-infected macaques] showed that stimulation of the immune system induces a temporary increase in the number of blood cells infected with the retrovirus^{51,52}. A similar effect was also seen in HIV-infected subjects administered with influenza vaccine. Vaccination was rapidly followed by a temporary (3–4 weeks) peaking of viral HIV RNA that subsequently returned to pre-vaccination levels^{53–55}. The magnitude of the increase in viral replication suggests that HIV is not only produced by infected T cells specifically stimulated by the vaccine but also by bystander T cells. Similar conclusions have been drawn from another study in which the variation of the viral load was measured following vaccination with tetanus toxoid, a recall antigen⁵⁶. In this case, increased HIV replication was associated with elevated expression of the activation marker HLA-DR on patients' lymphocytes.

However, we must not conclude from these studies that vaccination is detrimental to HIV-infected subjects. In the longer term, the benefit of vaccination seems to be much greater than the risk associated with a transitory elevation of HIV replication. Nevertheless, the effect of antiretroviral treatments given at the time of vaccination should be evaluated.

New antiretroviral treatments and restoration of a functional immune system

Recent antiretroviral treatments combining nucleoside analogues and protease inhibitors have been shown to increase the level of CD4 T cells significantly and, concomitantly, to decrease the viral load⁵⁷. It is still unclear whether the elevation of CD4 T cells results from peripheral lymphocyte multiplication or to cell renewal from naive progenitors. Ongoing studies aim to define whether these CD4 T cells are functional (by assaying their response to recall antigens, production of cytokines and activation state). In patients treated with the new antiretroviral regimens, markers other than CD4 T-cell counts and viral burden are needed to evaluate immune system function. Among these, soluble activation markers (such as the TNF- α receptor type II), membrane markers (such as CD28, CD38, HLA-DR and CD45RO), homing markers (such as CD62L) or apoptosis markers (such as Bcl-2, Fas and Fas-L) should be monitored. In addition, an evaluation of the variation in the TCR repertoire during antiretroviral therapy would help to understand the dynamics of the T-cell reconstitution.

Microbial cofactors and their contribution to disease progression

The influence of microbial infections on the clinical evolution of HIV-infected patients has generally been studied in mycobacteria. One of the hallmarks of the pathophysiology of mycobacterial infections is the production of large amounts of cytokines, including TNF- α , interleukin 1 β (IL-1 β) and interleukin 6 (IL-6), which are not only involved in defence against these infections but also stimulate HIV replication in monocytes/macrophages⁵⁸. Disseminated infections of *Mycobacterium avium* complex (MAC) are the most common form of systemic bacteraemia in AIDS patients, at least in the USA. The occurrence of MAC bacteraemia is related to the number of blood CD4 T cells, and the incidence of MAC increases markedly in patients with <100 CD4 T cells mm⁻³. The medical prognosis in such cases is poor, with a median survival of 130 days⁵⁹. In addition to the stimulation of cytokine production, MAC infections among AIDS subjects are associated with increased concentrations of β 2-microglobulin and plasma HIV p24 antigen. These observations suggest that MAC infections can act as cofactors of HIV to hasten the progression of the disease.

Progressive alteration of the immune system during HIV infection results in an increased risk of reactivation of a latent infection of *M. tuberculosis* or increased susceptibility to primary *M. tuberculosis* infection. In Africa, Asia and the poorer areas of industrialized countries, TB is the most common opportunistic infection among patients dying of AIDS. A study of HIV-positive

Questions for future research

- In addition to *Mycobacterium tuberculosis*, are there other microbial agents that are cofactors in HIV disease progression?
- What would be the benefit of anti-tuberculosis (TB) prophylactic treatment on HIV disease progression in countries with a high prevalence of TB?
- What is the long-term impact of temporary peaks of HIV replication induced by vaccination or short-term infections?
- Are there specific T-cell subsets responsible for the increased HIV load associated with co-infections? Do these cells produce HIV as a result of specific T-cell receptor ligation with cofactor antigens, or are they bystander cells activated by soluble factors?
- What is the influence of new antiretroviral therapies on the restoration of effective immune responses against AIDS-associated bacterial, viral and parasitic infections?
- In case of partial failure of antiretroviral therapies, would a combined cytokine-based immune therapy offer clinical and immunological improvement?

patients in the Ivory Coast revealed TB in 54% of cadavers with AIDS-defining pathology, whereas *Pneumocystis pneumonia* was found in only 4% of cadavers²⁶. African patients infected with *M. tuberculosis* show increased serum concentrations of TNF- α and β 2-microglobulin⁶⁰. Epidemiological studies have clearly shown that TB can accelerate the course of HIV infection^{13,14}, possibly by stimulating HIV replication. *M. tuberculosis* infection has been shown to increase the viral load in patients monitored before and during TB infection⁶¹. Moreover, in these patients successful tuberculosis treatment was associated with a reduction in viral load. Although the exact cellular and molecular mechanisms involved in the synergism between HIV and mycobacteria are not clear, independent studies have shown that *M. tuberculosis* (or extracts) can stimulate transcription of HIV genes and virus production from monocytic cell lines⁶¹⁻⁶³. In addition, circulating monocytes/macrophages obtained from patients with tuberculosis show an increased susceptibility to infection with HIV-1 *in vitro*⁶⁴. Analogous to the situation observed for HIV/TB co-infection, the onset of bacterial pneumonia (other than tuberculosis) has been correlated with an increase in plasma HIV RNA viral load⁶⁵. However, these observations were made on a small number of patients and the variation seen in viral load before and after pneumonia was not significant. A larger study would allow statistical evaluation of the influence of bacterial pneumonia on the virological and immunological status of patients with HIV.

In addition to acute bacterial infections, there is the possibility that chronic, clinically silent infections could sufficiently activate the immune system to elevate HIV replication. Mycoplasmas are possible candidates for such chronic infections. An interaction between mycoplasmas and HIV was first suggested to us by experimental data obtained *in vitro*, which showed that different species of mycoplasmas, including *Mycoplasma fermentans*, *Mycoplasma penetrans*, *Mycoplasma pirum* and *Mycoplasma arginini*, could increase the cytopathic effects associated with HIV replication^{66,67}. Other research groups have detected *M. fermentans* in various tissues of patients dying with AIDS (Ref. 68). In

addition, epidemiological studies indicate a higher seroprevalence of *M. penetrans* in HIV-infected subjects than in other groups, including blood donors and sexually transmitted disease patients^{69,70}. This recently described mycoplasma species has, so far, only been isolated from HIV-infected individuals, and its prevalence is strikingly similar to that of HHV-8, with high prevalence among HIV-infected homosexuals and low prevalence among other groups of HIV-positive subjects^{71,72}; however, *M. penetrans* does not seem to be statistically correlated with the development of Kaposi's sarcoma⁷⁰. The putative role of mycoplasmas as an HIV cofactor is now being evaluated in our laboratory by longitudinal epidemiological studies.

Conclusions

The identification of infectious cofactors in HIV disease is important because these agents are potentially amenable to treatment. Prophylactic antibiotic treatment has been shown to be beneficial for HIV-infected patients in industrialized countries. There is also an urgent need to provide TB prophylactic treatment to patients living in Africa and Asia. Co-infections have a tremendous impact on the survival of HIV-infected individuals with no access to medical care, such as those living in Africa and Asia, and those of low socioeconomic status in industrialized countries. Recent developments in antiretroviral therapies are very promising from the point of view of reducing viral load, but the functional restoration of the immune system has yet to be verified, as has the influence of new drug regimens on the prevalence of opportunistic infections.

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PrP genetics in sheep and the implications for scrapie and BSE

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Bovine spongiform encephalopathy (BSE) is a relatively new disease of cattle that has attracted a great deal of publicity recently. It is one of a group of related diseases known collectively as transmissible spongiform encephalopathies (TSEs), the earliest recognized of which is scrapie, which occurs in sheep and goats. The TSEs are all slowly progressive, inevitably fatal, neurodegenerative disorders that are characterized by vacuolated brain neurones and the deposition of an abnormal form of a host protein (PrP or prion protein). Most TSEs have also been shown to be experimentally transmissible. There is a strong genetic component in the

The strong links between PrP genotype and the occurrence of scrapie in sheep strengthen evidence supporting the central importance of the PrP protein in the development of transmissible spongiform encephalopathies, despite the fact that the cattle PrP gene has, so far, failed to show any association between PrP alleles and susceptibility to BSE.

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patterns of incidence of scrapie in sheep and of some forms of human TSE, and there is overwhelming evidence that the genetic component in these species is the PrP gene. Although polymorphisms and mutations of the PrP gene are linked to incubation period of experimental TSE and to the occurrence of natural TSE disease in mice, sheep, goats and humans, such linkage has not yet been demonstrated in cattle.

PrP consists of ~250 amino acids (exact length depends on the species), is glycosylated at either one, or both, of two possible glycosylation sites and is attached to the outside of the neuronal cell membrane by a glycosylphosphatidylinositol (GPI)