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KRISTER KRISTENSSON

NEURONAL TARGETING AND FUNCTIONAL EFFECTS OF INFECTIOUS AGENTS TRANSMITTED FROM ANIMALS TO MAN

ABSTRACT. — The nervous system is an «immune-privileged» site and can provide a reservoir to harbor as persistent or latent infections certain microbes that find their way to the brain. From an evolutionary standpoint, such infections are characterized at most times by low levels of the infectious agent in the systemic domain, except when multiplication has just taken place. Hence the ability for transmission of the pathogens from animals to Man will be determined by the availability of microbes to be transferred by a vector (*e.g.* in trypanosomiasis), or the amount of infective forms of the microbes shed into an environment (*e.g.* in toxoplasmosis). Using African trypanosomes, toxoplasma, *Listeria* and influenza A virus as examples, mechanisms by which microbes can spread and be targeted to and within the brain to cause various types of nervous system dysfunctions is reviewed. Newly revealed potentials of certain cytokines to stimulate neurons to control the growth, and even kill, microbes in their cell bodies is also described.

KEY WORDS: Brain; Infection; Virus; Bacteria; Parasites.

RIASSUNTO. — *Azione selettiva neuronale ed effetti funzionali di agenti infettivi trasmessi dall'animale all'uomo.* Il sistema nervoso è un sito «privilegiato dal punto di vista immunologico», che può quindi rappresentare un ricettacolo nel quale possono annidarsi, mediante infezioni persistenti o latenti, alcuni microbi. Tali agenti infettivi possono in tal modo avere accesso all'encefalo. Da un punto di vista evoluzionistico, queste infezioni sono per lo più caratterizzate da bassi livelli sistemici dell'agente infettivo, ad eccezione delle fasi immediatamente successive alla sua moltiplicazione. Da ciò deriva la trasmissibilità di patogeni dagli animali all'Uomo, che verrà determinata dalla possibilità di trasmissione di tali agenti tramite un vettore (come avviene, ad esempio, nella tripanosomiasi), o dalla quantità di forme infettive di microbi sparsi in un determinato ambiente (come si verifica, ad esempio, nella toxoplasmosi). Utilizzando come esempi il tripanosoma africano, il toxoplasma, la *Listeria* ed il virus influenzale A, questo contributo passa in rassegna i meccanismi tramite i quali i microbi possono diffondersi all'encefalo e all'interno del parenchima encefalico, causando vari tipi di alterazioni funzionali del sistema nervoso. Vengono inoltre qui riportati dati recenti, che attestano come alcune citochine abbiano la possibilità di stimolare i neuroni, rendendoli capaci di controllare la crescita di microbi e persino di ucciderli all'interno dei loro stessi corpi cellulari.

Animals are the natural reservoir for a large number of microbes that can be transmissible and infectious for Man (zoonoses). The awareness in the society of zoonoses flares up from time to time, and recent concerns involve risks of animal-derived pathogens in xenotransplantation and biological warfare, and the transmissible spongiform encephalopathies. Emerging and re-emerging zoonoses are constant threats to our brains, since microbes of different categories can spread from animals to the human brain to cause nervous system diseases. These include those of parasites (*e.g.* trypanosomiasis, toxoplasmosis), bacteria (*e.g.* Lyme disease, listeriosis), viruses (*e.g.* influen-

za, West Nile encephalitis, Borna disease, rabies, Japanese encephalitis, SARS), and prions (*e.g.* bovine spongiform encephalopathy, variant Creutzfeldt-Jakob disease). While the detection of the Negri bodies in rabies marks the beginning of the 20th century, the threat of a prion epidemic spreading from cows to humans has created a wave of anxiety at the end of the century. Prion infections are covered in the lecture by A. Aguzzi in this symposium, and this presentation will focus on transmissible diseases caused by the other categories of pathogens. These pathogens will be exemplified by African trypanosomes, *Listeria monocytogenes* and influenza A virus, which have been the subject of experimental studies in our laboratory, and by *Toxoplasma gondii*, which in spite of its high prevalence in the human population (10-25% of the world's population), seems to have been largely neglected in neuroscience research. The choose of these infections is also given by the fact that they illustrate how microbes can use different pathways for spread and targeting to and within the brain as well as how they can use different strategies to interact with neurons to cause degeneration or functional disturbances of the host brain.

Spread of African trypanosomes across the blood-brain barrier

By excluding most macromolecules from passing into the brain, the blood-brain barrier (BBB) also prevents most microbes from entering the nerve parenchyma. In spite of this, a number of pathogens have adopted mechanisms for a hematogenous spread to the brain. For instance, *Toxoplasma gondii* are carried by macrophages in the blood. From these macrophages they can be released to infect and multiply in brain endothelial cells for further passage into the nerve parenchyma. Other pathogens, *e.g.* rabies virus, spread instead to the central nervous system along peripheral nerves and some, *e.g.* influenza A virus and *Listeria monocytogenes*, may spread both via the blood and along peripheral nerves as will be discussed in a following section.

We have focused our interest on mechanisms by which subspecies of the extracellular parasite *Trypanosoma brucei* (*Tb*) cross the BBB. *Tb* causes African trypanosomiasis (sleeping sickness), which is an example of a re-emerging zoonosis. Although the disease in humans, Human African Trypanosomiasis, had almost disappeared in the early 1960ths, the incidence of the disease has now almost returned to the same levels as in the 1920ths. The African trypanosomes spread by the tsetse fly in sub-Saharan Africa and use both wild game and domestic animals, *e.g.* cattle and pigs, as reservoirs. The subspecies *Tb rhodesiense* in East and Southern Africa causes a relatively rapid disease, while *Tb gambiense* in West and Central Africa causes a more protracted disease hallmarked by disturbances in sleep patterns. Infections with both subspecies lead invariable to death when left untreated. For therapeutic purposes, the question of how and when the parasites pass the BBB during the course of disease has important consequences. At an early stage of the disease relatively non-toxic drugs, which do not pass the BBB, are available, but for the late stage with involvement of the nervous system arsenic preparations are the drug of choice. These arsenic compounds are

trypanocidal and pass the BBB, but they are also neurotoxic and cause lethality in a high proportion, up to 10 %, of the patients. The diagnosis of the late stage relies on the finding of parasites and/or white blood cells in the cerebrospinal fluid. The mechanisms of trypanosome entry into the brain are still not known, and the proper staging of the disease, *i.e.* determination of the stage when the parasites has passed beyond the BBB, remains to be clarified. With the aim of determining how and when trypanosomes pass the BBB, we are undertaking a series of studies on invasion of the rodent-pathogenic *Tb brucei* strain into the brains of mice and rats. In both animals, the trypanosomes localize early during infection to regions in the brain that lack a BBB, *i.e.* the choroid plexus and the circumventricular organs. At later stages, however, we have recently found that the parasites can penetrate into the brain parenchyma through the cerebral vessels (fig. 1), although the tight junction proteins of the endothelial cells are preserved (Mulenga *et al.*, 2001). This indicates that the penetration of trypanosomes into the brain parenchyma is an active process and not a consequence of a damaged BBB. We have obtained preliminary evidence that the pro-inflammatory cytokine, interferon (IFN)-γ may be involved in regulating the neuroinvasion. In IFN-γ receptor knock out mice, the parasites stay in or around the cerebral vessels and do not spread into the neuropil (Kristensson *et al.*, unpublished observation). The mechanisms by which IFN-γ may facilitate invasion of the trypanosomes may involve effects on the brain tissue or on the trypanomes. For instance, this cytokine is a most potent inducer of expression of chemokines in astrocytes and microglial cells in the brain and of proteases, and may regulate cell junctions as well. Such factors are involved in attraction and penetration of different populations of white blood cells across the BBB, and may

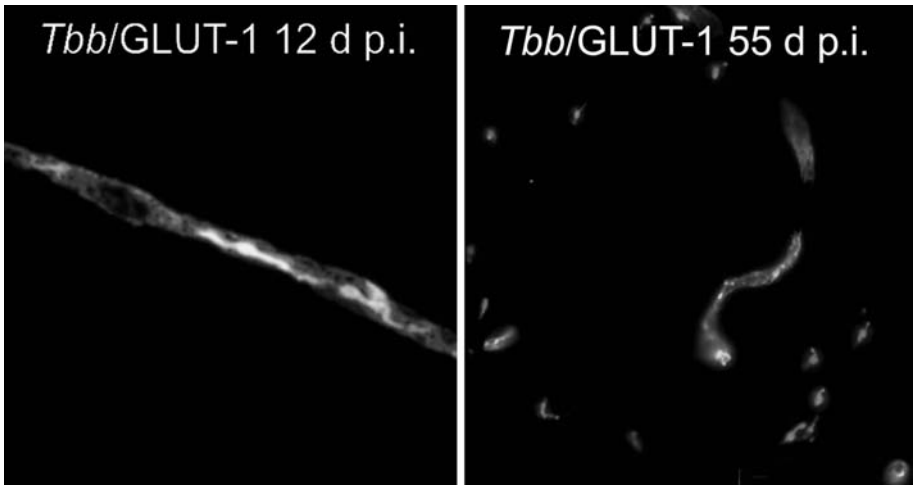

Fig. 1. – Invasion of African trypanosomes (*Tb brucei*) through the blood-brain barrier. Brains from rats sampled at 12 (left) and 55 (right) after intraperitoneal injection of the parasites. Double-immunolabelling of the variable surface glycoprotein of the parasites (red) and glucose transporter-1 of the cerebral endothelial cells (green). At 12 days after infection, the parasites are confined to the vessel lumens, while at 55 days after infection they have invaded the brain parenchyma.

hypothetically therefore facilitate the invasion of trypanosomes. The trypanosomes may have an advantage to pass through an intact BBB into the brain, because this would protect them from neutralization by circulating antibodies. In fact, treatment of mice with drugs that do not pass the BBB can clear the infection from the organism except from the brain, where the parasites have been suggested to persist for extended periods to cause late relapses (Jennings *et al.*, 1979). The most characteristic clinical feature of nervous system involvement by the parasites is the sleep disturbances manifested as a disruption of the sleep pattern and circadian rhythms. In collaboration with Prof. M. Bentivoglio we have described marked dysfunctions of the suprachiasmatic nucleus, which is the main pacemaker for circadian rhythms, in an experimental model of the disease. These findings have been reviewed elsewhere (Bentivoglio *et al.*, 1994; Kristensson *et al.*, 2002).

Neurodegeneration induced by sodium salicylate during trypanosome infections

Trypanosome infections evoke a marked inflammatory response in the brain, which in humans is most intense in the white matter; a leukoencephalitis. During this process, inflammatory cytokines are induced in astrocytes and microglial cells (Hunter *et al.*, 1992; Quan *et al.*, 2000). In spite of the long-standing induction of proinflammatory cytokines, which potentially may be neurotoxic, there are only minor signs of neurodegeneration in trypanosome-infected brains, both in human clinical materials and in experimental rodents (Kristensson and Bentivoglio, 1999). This is interesting in view of the fact that over-expression of inflammatory cytokines have been implicated in the pathogenesis HIV dementia and in neurodegenerative diseases, such as Alzheimer's disease. Many of these cytokines are activated by nuclear factor-kB (NF-kB). Aspirin and its metabolite sodium salicylate inhibit NF-kB-initiated transcription of immune response genes and treatment regimes with this drug have been considered in neurodegenerative diseases. Since a chronic rodent infection with trypanosomes would provide an *in vivo* model of long-term inflammatory neurodegeneration, we have examined the effects of chronic treatment with sodium salicylate on neurodegeneration and cytokine RNA expression in trypanosome-infected rats. In brains from untreated trypanosome-infected rats, degeneration, as detected by a modified cupric silver stain, was limited to certain nerve fibers in the vagus, lateral olfactory tract and some other white matter tracts, while sodium salicylate treatment resulted in extensive terminal and neuronal cell body degeneration in the cortex, hippocampus, striatum, thalamus and anterior olfactory nucleus. This exaggerated neurodegeneration was temporally and spatially associated with enhanced mRNA expression of interleukin-1b, interleukin-1b converting enzyme, tumor necrosis factor-a, and inhibitory factor kBa in the brain. Restricted areas showed elevations in mRNA expression of interleukin-1 receptor antagonist, interleukin-6, inducible nitric oxide synthase, IFN-γ, and inducible cyclooxygenase (Quan *et al.*, 2000). Our results reveal an

unexpected, serious complication in using aspirin drugs for treatment of chronic inflammatory brain conditions.

SPREAD OF *LISTERIA MONOCYTOGENES* ALONG PERIPHERAL NERVES

In contrast to the effect of IFN-γ in facilitating invasion of the extracellular African trypanosomes into the brain parenchyma, we have found that the same cytokine plays a crucial role in preventing spread of the intracellular bacterium, *Listeria monocytogenes*, to the brainstem along the trigeminal nerves. *Listeria* is widespread in nature and can infect several animal species. The bacteria pose a particular problem in sheep farming. They cause the so-called «circling disease» in sheep, which is a lethal disease with signs of brainstem encephalitis. By electron microscope, *Listeria* has been found within axons of the trigeminal nerve in infected sheep (Otter and Blakemore, 1989). An encephalitis similarly targeted to the brainstem has, since its first description by Eck (1957), been reported in hundreds of human patients and the disease has commonly followed the eating of *Listeria*-contaminated, unpasteurized cheese (Armstrong and Fung, 1993). For study of factors that control spread of *Listeria monocytogenes* along the trigeminal nerve to the brainstem, we have developed a mouse model in which the bacteria are injected into the snout of different immuno-deficient mice and the appearance of the bacteria in neurons of the trigeminal ganglia and brainstem recorded. In recombination activating gene 1 (*RAG*-1)-deficient mice a neural route of infection was suggested after snout injection of the bacteria (Jin *et al.*, 2001). This suggestion was based on immunostaining of *Listeria monocytogenes* in the trigeminal ganglia and brainstem, but not in other areas of the brain; the kinetics of bacterial loads in snout, trigeminal ganglia and brain; and the increased resistance of mice infected with the *plcB* bacterial mutant (unable to spread from cell to cell). By using mice genomically lacking the mononuclear phagocytic growth factor colony-stimulating factor 1, and thereby deficient in macrophage and dendritic cell populations, we found that these cells play a dual role in *Listeria* infections (Jin *et al.*, 2002). On the one hand, they constitute a major defense against systemic infections, but on the other hand, they facilitate the invasion of the bacteria along the trigeminal nerve after snout injection. Since dendritic cells of the $CD11c^+$ phenotype are in direct contact with peripheral nerve fibers, it may be suggested that these cells serve as an amplifier of *Listeria* replication and thereby increasing the chances of bacterial spread to nerve fibers, which is dependent on direct cell contacts (fig. 2).

IFN-γ plays a protective role against *Listeria* neuroinvasion along the trigeminal nerve and inducible nitric oxide synthase accounts partially for this protection. This was shown by comparison of the susceptibility to infection by *Listeria monocytogenes* in the snout of mice deleted for the genes of both the IFN-γ receptor and the inducible nitric oxide synthase with wild-type mice. The source of IFN-γ appeared to be natural killer cells as shown by the use of combined *RAG-1*-deficient, γ-chain receptor gene-deficient mice. *In vitro* experiments showed that IFN-γ can directly inhibit growth of *Listeria* in dorsal root ganglia cells (Jin *et al.,* 2001), and recent experiments ha-

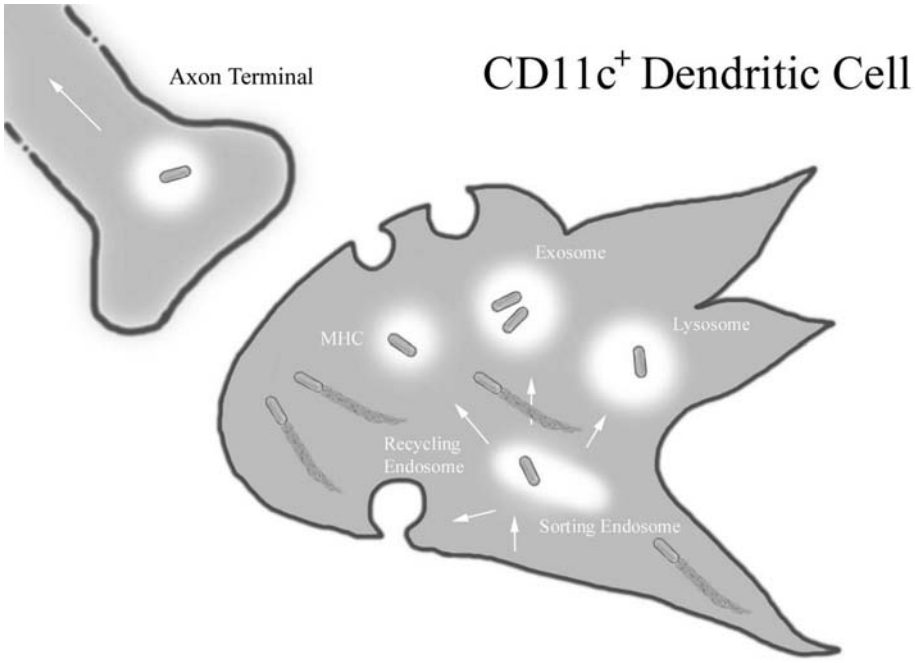

Fig. 2. – *Listeria monocytogenes* multiplies first in macrophages and/or dendritic cells before they invade peripheral nerve fibers for retrograde axonal transport to cell bodies in trigeminal ganglia neurons.

ve shown that IFN-γ can in fact even induce the killing of bacteria within neuronal cells (Jin *et al.*, unpublished observations). Taken together these observations show that *Listeria monocytogenes* after initial replication in macrophages/dendritic cells have the propensity to infect peripheral sensory neurons and spread to the central nervous system. At the same time, however, sensory neurons can control the replication of *Listeria* and IFN-γ may induce neurons to kill bacteria. This observed new function of a neuron, *i.e.* a bacterocidal activity, may be of relevance for an understanding of how spread and replication of infectious agents can be controlled in the immuno-priveledged nervous system.

Effects of *Toxoplasma gondii* on brain functions

During infections with the parasite *Toxoplasma gondii*, direct effects of IFN-γ on replication of a pathogen in somatic cells have also been observed. This parasite replicates rapidly in macrophages, and IFN-γ activation of these cells was previously considered as the major effectors for resistance to this intracellular pathogen. However, recent studies employing experiments on chimeric mice have shown that also nonhemopoietic cells can directly mediate IFN-γ-dependent resistance of the host during *Toxoplasma gondii* infections. In fact, it was speculated that control of intracellular parasitic replication is a reason why the IFN-γ receptor is retained in nucleated somatic cells

(Yap and Sher, 1999). After infection of neurons (and skeletal muscle cells), the growth of *Toxoplasma gondii* in a host is much retarded and the parasites persist in the brain as slowly replicating bradyzoites (Fagard *et al.*, 1999; Lüder *et al.*, 1999). Also in the nervous system, growth of toxoplasma may be under the control of IFN-γ (Fagard *et al.*, 1999), although the precise role of the cytokine remains to be determined (Schlüter *et al.*, 2001). In spite of the long-standing infections with release of cytokines that control the intracellular growth of the parasites there are, like in African trypanosome-infected brains, no or only minor signs of neurodegeneration. The question how potentially neurotoxic cytokines can control growth of the parasite in neurons without causing their damage has been addressed in cell culture systems. Both astrocytes and neurons are infected by the toxoplasma parasites, but supernatant from infected astrocytic cultures could prevent infected neuronal cultures from degeneration (Rozenfeld *et al.*, 2003). The suggestion of a neuroprotective effect of astrocytes during infections is accordance with our studies showing such an effect in mumps virus-infected hippocampal neurons (Owe-Larsson *et al.*, 1997). These studies emphasis that infections in the brain are controlled not only by molecules that arrest intraneuronal microbe growth, but also by molecules that may protect neurons from damage. Since microbes, like toxoplasma, may persist in the brain for the life span of the host, the questions arise whether the balance in release of microbe growth control and neuroprotective molecules can be altered to cause neurodegeneration later in life, and whether the infected neurons or the release of the molecules have any effect on neuronal function and behavior of the host.

The latter question, *i.e.* potential behavior consequences of latent toxoplasma infections in the brain, was recently addressed by Berdoy *et al.* (2000). The definite host animal of *Toxoplasma gondii* is the cat, while rodents, birds and by accident humans are intermediate hosts (fig. 3). Although the brain provides a protected site for hiding of the parasite, it may also be a cul-de-sac: if the intermediate host dies, the parasite will die, unless the predator eats the intermediate host. According to the hypothesis of parasite manipulation of host behavior, a parasite may alter the behavior of a host to favor its transmission to another animal (Poulin, 1994). Rats fear the smell of urine of their predator, the cat, but not that of a rabbit. However, this fear was lost in toxoplasma-infected rats; the infected rats were even attracted to the cat urine, and this «fatal attraction» will increase the chances of predation (Berdoy *et al.*, 2000). In a review of human latent toxoplasma infections, it was noted that infected children may have a somewhat reduced IQ, and that both men and women may have certain traits in their personality profile (Webster, 2001). Infected men had a higher tendency to disregard rules of the society, they were more suspecting, jealous and dogmatic, while infected women were more warm-hearted, out- and easy-going, but also more conscientious, persistent, moralistic and staid (Flegr and Hrdá, 1994; Flegr *et al.*, 2000). Whether these findings are reproducible in other studies and societies remains to be evaluated as well as whether they represent causes or consequences of the infection. However that may be, these very common infections of the brain deserve studies in more detail.

Fig. 3. – Since toxoplasma parasites can be transmitted to the human brain and cause nervous system dysfunctions, contacts with infected the host animal (cat) should be avoided especially during early life.

Persistence of influenza A viral RNA in the mouse brain

Another common infectious agent transmissible from animals to Man, which has been associated with human behavior disturbances is influenza A virus. There are well-documented cases of transmission from pigs to humans, and a few years ago direct spread of influenza A virus from hens to humans caused an epidemic in Hong Kong. Pigs, ducks and humans are considered as a melting pot for this virus, and since the virus is segmented, reassorted variants can appear. Thus, there is always the threat that new variants with neurotropic properties may araise. Even in current epidemics, encephalitis due to influenza is not uncommon; in a recent study of 3,231 patients in Finland (population 4 million) with acute central nervous system disease of suspected viral origin collected during 1995-1996, varizella-zoster virus was the main cause of disease followed by herpes simplex virus, enteroviruses and influenza A virus (Koskiniemi *et al.*, 2001). There are anecdotal reports that psychiatric disturbances followed influenza in past epidemics; for instance, it was said that the world was never the same again, mentally, after the 1890 epidemic, and a reversible schizophrenia-like syndrome was associated with the 1918/19 epidemic (Menninger, 1926). There are also a number

of studies, although the data are equivocal, suggesting an association between influenza A virus infections during pregnancy (the second trimester) and schizophrenia. Although, taken together the epidemiological studies seem to have shown such an association, these type of studies may not contribute more than to indicate a relationship and the «*the initiative now rests on cellular anatomy and neurophysiology*» (Munk-Jørgensen and Ewald, 2001) to forward our knowledge.

In order to study the potentials of influenza A virus to cause developmental and/or functional disturbances of the nervous system, we have used a mouse-neuroadapted strain, WSN/33, of influenza A virus, which is derived from the first influenza a virus isolate 1933, in a series of studies in mice. This virus causes a lethal disease in mice with virus targeting to neurons in the hippocampus and *substantia nigra* following intracerebral or systemic injections (Takahashi *et al*., 1995). Following injection into the olfactory bulbs, the virus spread to the anterior olfactory, the midline thalamic and medial habenular nuclei as well as to the ventral tegmentum areas. Following this way of injection, the mice survive and the virus is cleared from the brain (Mori *et al.*, 1999). Our preliminary studies have shown that these surviving mice show certain disturbances consisting of learning disabilities and altered behavior in an elevated plus maze test (Aronsson *et al.*, unpublished data). In immunodefective transporter associated with antigen processing 1 (TAP1) mutant mice similarly infected with the virus, all segments of the genome of the virus could persist in the brain for more than 17 months after infection. Viral RNA encoding the nonstructural NS1 protein was detected in sections from the brain at midbrain levels by RT-PCR in almost all animals. Both negative-strand genomic RNA (vRNA) and positive-strand RNA, including mRNA, were found (Aronsson *et al.*, 2001). This observation shows that certain regions of the brain in immunodefective mice may harbor the genome of influenza A virus, including the NS1 gene the products of which may play a regulatory role in a host cell metabolism.

In order to investigate if maternal influenza A virus has the potential to cause brain dysfunctions in the offspring, the WSN/33 strain was instilled intranasally into mice at day 14 of pregnancy (Aronsson *et al.*, 2002). When the fetuses were examined 3 days later, viral nucleoprotein and RNA were detected in their brains. After showing that influenza A virus could pass the placenta and be targeted to the fetal brains we studied in another series of experiments the effect of the maternal infection on the offspring. At an infectious dose of 7,500 plaque forming units (PFU) intranasally instilled during pregnancy, mice were born but all died within 2-8 days after birth. When the viral dose was reduced to 750 PFU, all offspring survived. About 25 % of brains sampled from these offspring at 10, 20, 35, 60 and 90 days after birth showed presence of viral RNA encoding the matrix and nucleocapsid proteins by RT-PCR (fig. 4). These studies show that influenza A virus can persist in the brain even of non-immunocompromised individuals after an infection at early life. In our preliminary studies, we have observed that there are certain changes in the gene expression profile, verified by real time PCR, in brains from the offspring. Interestingly some of these changes did not appear until 90 days of age, which may indicate that a maternal influenza A virus

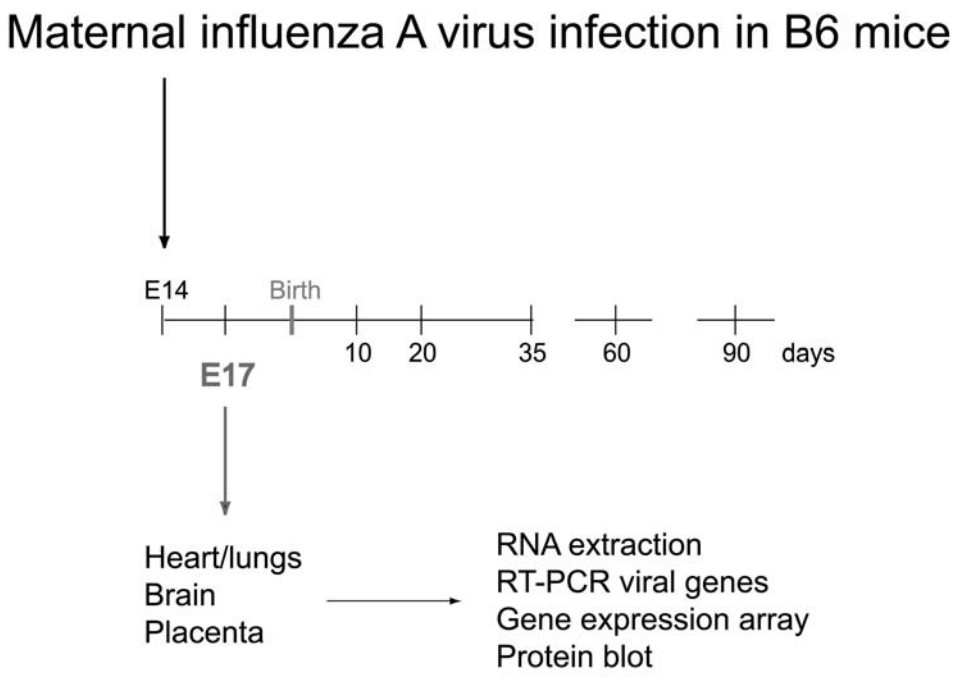

Fig. 4. – Influenza A virus instilled intranasally in pregnant mice can be transmitted to the fetal brains and persists in the brains of the offspring for at least 90 days after birth. The gene expression profile of the brains shows differences between offspring to infected mice and uninfected, control mice.

infection can cause gene expression changes that progress over time to become manifested later in life.

Concluding remarks

New observations on the neuropathogenesis of infectious agents that can be transmitted from animals to Man have been highlighted and these include: *i*) The role of cytokines for spread and targeting to the brain. While the host derived inflammatory molecule IFN-γ seems to inhibit the invasion of the intracellular *Listeria monocytogenes* along trigeminal nerves, it had the paradoxical effect of facilitating entry of the extracellular African trypanosomes into the brain through the BBB. *ii*) Populations of neurons that are attacked by pathogens have the capacity to control growth, and even to kill, the microbes. This occurs by mechanisms that at the same time protect neurons from being damaged. Pharmacological treatment may tilt a balance between potentially neurotoxic and neuroprotective molecules in an unpredictable direction to cause severe neurodegeneration in the brain. *iii*) Microbes may induce imbalances in neuronal circuits and gene expression changes that may progress in later life. Questions may therefore be addressed whether microbes transmitted from animals could be included

in the list of potential causes of behavior disturbances or even neuropsychiatric diseases in Man.

Acknowledgements

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Department of Neuroscience
Retzius väg 8
Karolinska Institutet
SE-171 77 STOCKHOLM (Svezia)
krister.kristensson@neuro.ki.se