Interferon and Aicardi-Goutieres Syndrome

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I would like to thank the organisers of this meeting for their hospitality, and I thank them especially for inviting me to participate in this interesting congress; I thank in particular Mrs Boni Longo, professor Lanzi, doctor E Fazzi and also doctor Jean Aicardi whom I have the pleasure to meet again here.

Marco De Andrea has spoken at length about interferon, and I would like to show you only some data concerning the relationship between interferon and the Aicardi-Goutières Syndrome.

There are two types of interferon (IFN), as you know already, and the interferon which we are interested in for this syndrome is type I IFN, specifically the alpha type. Interferons are proteins produced by the host in response to viral infection. Interferon alpha and beta (type I) are not normally detected in blood of healthy people, but they are found mostly in different specimens of patients with viral infections, such as blood, cerebrospinal fluid, vesicular fluid etc..

**Interferon at the cellular level**

Naturally, IFN genes are repressed, and there is only a slight constitutive expression of interferon (without inducers), which does not lead to a detectable amount of the corresponding protein in the blood. The main cells in blood that produce interferon-alpha are pDC2 cells (Siegal) precursors of dendritic cells, which are very efficient in producing interferon-alpha in response to various viruses. This population is very rare in the blood; it represents one cell per 1000 white cells. However, other populations of leukocyte cells (monocytes, B cells) can also produce interferon in some conditions, and their involvement depends on the nature of the inducer (For example B cells and Epstein Barr Virus (EBV)).

Double-stranded RNA was the first molecule implicated in the mechanism of induction of Type I IFN, but it is an inducer primarily for the beta and only slightly for the alpha type. Some viral glycoproteins, for example herpes virus glycoprotein D (Ankel 1998) or HIV gp120 (Ankel 1996) induce interferon-alpha in vitro; under specific conditions some bacteria have also been shown to induce small amounts of type I IFN (Svensson) in Sweden. However, in vivo we have not detected interferon-alpha in specimens with bacterial infections except in some very rare cases. Picornaviruses bound to antibodies can also stimulate alpha interferon synthesis (Palmer). These viruses in the absence of antibodies induce only a weak alpha-interferon response. Some chemical substances, such as Imoquimod, are interferon inducers, and this substance is tried in some dermatological diseases. Probably, interferon-alpha could be produced in other situations and we will discuss this point later.
Interferon and viral diseases

The amount of IFN-a that we consider to be normal in blood, is less than two international units per millilitre (IU/ml) measured with a biological assay.(Gresser1974).

Interferon alpha is detected in acute viral diseases at different levels, which are related to the stage of the disease, where levels between 2 and 5000 IU/ml can be detected.

As it was previously shown, in experimental infections with influenza virus, we have observed an early peak of interferon in the blood of volunteers. Indeed, in peripheral infections like influenza, but also in digestive infections by rotavirus, a very strong production of interferon-a can be measured in the blood, although there is no viraemia during these infections. IFN producing cells that spread from the initial site of infection could explain this circulating IFN.

Naturally, IFN-a is also produced in systemic infections, such as measles, mumps, AIDS, enteroviral infections (Deboissieu), but also in congenital infections with cytomegalovirus or rubella virus. In congenital rubella virus infection, the synthesis of blood interferon-a starts during the 20th week of pregnancy (perhaps sooner) (Lebon1985) and continues until birth, since sometimes infants chronically infected with this virus secrete interferon alpha.

Interferon and viral infection of CNS

Interferon-alpha is also produced in the central nervous system. The normal value in cerebrospinal fluid is also fewer than two units per ml. It is a main feature of interferon-a that it does not cross the intact blood-brain barrier, and when 100 units/ml of interferon-a are released in the blood, only one or less than one unit /ml is detected in the CSF of patients. Therefore, when IFN-a is detected at similar or higher titres in CSF than in serum, we can conclude its intrathecal synthesis.

Because many neuropediatricians like Jean Aicardi, Françoise Goutieres, J Arthuis, Gerard Ponsot (also Gilles Lyon before 1980) stayed at St.Vincent de Paul Hospital for a long time; we were attracted to the field of encephalitis and other viral infections of the central nervous system. With them, we demonstrated that patients with primitive encephalitis, especially with herpes encephalitis(Lebon1979), early produced interferon-alpha and (also gamma) in the cerebrospinal fluid, which disappeared rapidly six to eight days after the initial symptoms. IFN-a is also detected in CSF of patients with HIV encephalitis (Krivine1999) or with meningitis generated by mumps virus, enterovirus and varicella zoster virus, and also in newborns with congenital rubella and neurological symptoms, (Dussaix1985). An exception to the CNS viral infections is lupus, which is associated with the secretion of alpha interferon during the attack of the neuro-lupus(Lebon 1983). In this case there is no account for its induction in the CNS.

Interferon–a is not synthesized in all the neurological diseases, and it is very uncommon to find interferon in non-viral diseases. We have observed that in contrast to primitive encephalitis, in post-infectious encephalitis such as measles encephalitis, interferon is not detectable in the cerebrospinal fluid. of patients with Guillain-Barré syndrome, in multiple sclerosis, in Creutzfeldt-Jakob disease, and in bacterial infections (Raymond1992), except in a few cases of Listeria encephalitis.

We have tested many cerebrospinal fluid and serum samples from patients with convulsive encephalopathy, infantile spasms, cryptogenic mental retardation, two cases of Cockayne’s syndrome, two cases of Krabbe syndrome: in these metabolic diseases interferon-alpha is not implicated( unpublished data).
Interferon and AGS

As you already know, interferon-alpha (IFN-a) is involved in AGS. In 1982 we discovered the presence of IFN-a in a newborn girl affected with a fetopathy-like syndrome. We did not know at this time that it was a case of AGS. She had a high level of interferon-a in her cerebrospinal fluid and in her serum. We could not isolate any virus or TORCH agents in various specimens from her. Four years later and after the birth of a normal sister, a brother was born with the same symptoms and with the same high level of interferon-alpha. At this time in 1986, while discussing with G. Ponsot the observations of Jean Aicardi and Françoise Goutières (1984), we were able to associate this syndrome with an abnormal production of interferon alpha in CSF and blood (Lebon 1988). Until now we have tested 80 children, including two cases of Cre eencephalitis(Black1988) and one case of MICS syndrome (Reardon 1994), two genetic syndromes that are clinically similar to AGS (Lebon1996). As shown in table 1 between 1986 and 2001 we found IFN in 77 of 81 tested children, and we have 71 families registered. Among them, 18 families have consanguineous parents.

The typical feature of this interferon-a release in AGS is, that it occurs over a long time period, and I will present to you three children, whose interferon was measured at different ages. For the child in figure 1 the IFN-a level in CSF is higher than in serum at birth; but with age, the levels in CSF and serum become similar.

**Fig. 1.** In this child the interferon-alpha level in CSF was higher than in serum at birth but with age the levels in CSF and serum became similar.

**Fig. 2.** The interferon-alpha in CSF in this child decreased and stabilized at 9 years at the same level as in the blood.

**Fig. 3.** In this child a strong decrease of the interferon-alpha titres of blood and CSF occurred until 5 years of age, when interferon-alpha levels were negative, whereas at 6 years of age interferon was again found in the CSF and serum. These curves demonstrate that the synthesis of interferon-alpha is intrathecal but also systemic.

**Fig. 4.** Interferon titres in the CSF of 12 patients. Evolution of values with age in Aicardi-Goutières syndrome.
In figure 2 the production of interferon-a in CSF decreases and stabilizes at nine years at the same level as in the blood; in Figure 3, a strong decrease of the IFN-a titres of blood and CSF occur until 5 years, when IFN-a levels were negative, whereas at six years we find again interferon in CSF and serum. These curves demonstrate that the synthesis of IFN-a is intrathecal but also systemic.

In Figure 4 we show interferon titres in the CSF from 12 patients for which we could test two or more specimens. Some patients display the same IFN-a level in CSF one year later, but for most of them, IFN-a levels decrease slowly with time, similarly to the white cell decrease in CSF that Jean Aicardi showed you this morning (Goutieres1998). Low levels of interferon-a are frequently maintained after the age of 3 to 4 years.

| Table 2. Interferon-alpha titres and age at onset of Aicardi-Goutières syndrome |
|---------------------------------|-----------------|-----------------|
| Interferon-alpha titre mean     | (No.) | CSF  | Blood |
| Early onset: < 1 month          | 130   | (7)  | 20    |
| Measured in the first month     | (8)   | 66   | (13)  |
| 3 months < measured < 3 years   | 18    | 66   | (35)  |
| Late onset: > 3 months          | (25)  | 30   | (14)  |
| 3 months > measured > 3 years   |       |      | 6     |

CSF: Cerebrospinal fluid

If we consider the level of interferon-a in CSF and blood according to the onset of the disease, the averages of interferon levels appear to be very high in patients with early onset, whilst they are lower when the disease begun after 3 months. In the early onset form, the IFN-a levels in blood seem to decrease less rapidly as the level in CSF. (Table 1)

We have verified that the antiviral effect observed in specimens of patients with AGS is neutralized in vitro with monoclonal or polyclonal antibodies to interferon alpha. Furthermore, this antiviral activity is not neutralized by antiserum to interferon beta or gamma. However, it is difficult to ascertain that interferon beta is not present in the specimen, because specific tests for its detection are not available. It is possible that the interferon beta gene, which is very close to the alpha gene on chromosome 9, is also activated in this syndrome.

Research of a virus in AGS

Since the symptoms of AGS are reminiscent of those of viral congenital infections, at first we looked for viruses in this syndrome. We have checked, unsuccessfully, for many viruses in the serum and CSF of patients: We have attempted to isolate viruses from blood and from CSF in standard viral culture conditions or in conditions to isolate retroviruses like HTLV1 or HIV1. We have also inoculated some animals: nude mice that carry an immune defect, mice without the receptor for interferon alpha. We have also inoculated patient CSF into squirrel monkeys which are small monkeys, by intracerebral route with the help of Doctor Court, and we have observed these animals for over one year. All the results of serology, culture or inoculation were negative excepted for one child, who excreted CMV in its urines, and for one child of another family who carried HBS antigen in his blood.

We have also looked for the genome of herpes viruses, and we have shown only one positive reaction for Epstein-Barr virus in one patient out of 6 using a multiplex PCR (Rozenberg1991).
However, we believe that in this case EBV was the result of a superinfection, since it was not present, when we tested the child at birth. Both children of this family had high level of Epstein-Barr virus antibodies (Lebon1988).

Other biological features of AGS
We have tested some patients for the simultaneous presence of gamma interferon, TNF alpha and interleukin 8 and found that the level these cytokines did not rise. This discrepancy with the increasing of IFN alpha is an other argument for a unique immune response unrelated to a viral infection response.

We have tested cells from several series of patients for their sensitivity to interferon alpha, and we have detected no anomaly in the response to interferon-a in terms of its antiviral effect. We have also looked for a UV hypersensitivity in two families. When we treated the cells with different doses of UV we did not induce interferon alpha and the capacity of the cells to produce interferon upon virus infection remained similar in patients and in controls from the same family (Data not shown).

We have also assayed interferon in supernatants of cultures of peripheral blood mononuclear cells after infection by different viruses (herpes virus, Sendaï), or the interferon in the supernatant of cultured cells immortalised with Epstein-Barr virus; there is no evidence of a hyperproduction of interferon-alpha in the patients in comparison to the other members of the family.

I have already mentioned lupus: the interferon alpha in lupus patients is associated with the presence of an inducing factor of interferon in the serum. This was demonstrated in Sweden by Vallin(1999) al and by us (Batteux 1999)at the Hôpital-Saint Vincent de Paul. Such factor was neither present in the cerebrospinal fluid nor in the blood of AGS patients( data not shown).

Discussion:
This morning we have already talked at length about this syndrome and its relation to Cree encephalitis. The final proof that both syndromes and other phenotype of AGS (Kumar1998)(McEntagart1998) could represent the same disease must await the results of genetic studies.(Crow2000)

-Induction of interferon-alpha in AGS
The crucial question about AGS is: what induces interferon-a? Several hypotheses can be formulated: -It could be a direct induction, but no virus is involved in the disease.
-It could be a genetic defect in interferon regulation. The interferon regulating genes are very complex. Many interferon-regulating factors IRF 1 to 9 (Mamame 1999) are involved, but also many co-factors that influence the positive and negative control of the interferon-a genes. New genes concerning this regulation are discovered each year. Evidently, we survey the reports of such new genes to relate them with the AGS 1 locus or other loci suspected in the genetic study (Faure 1999).
-It could also be a deficiency in cytokines or in the receptor of cytokines that influence the synthesis of interferon-a. An example is interleukin 10 which inhibits the production of interferon-a in
PBMC. In one hypothesis it is thought that a deficiency of receptors for this cytokine could result in elevated production of interferon alpha as compared to controls. IFN-a in AGS could also be the result of an indirect induction associated with an immunity defect. As you know, the Cree children are very often infected with bacteria or some viruses that are resistant to interferon, such as herpes virus or Epstein-Barr virus, so that a chronic infection with a different infectious agent could produce a secondary induction of interferon. However, as you have heard, the isolation of superinfecting viruses is rare in this syndrome. So I believe that this hypothesis is not very plausible.

The hypothesis of endogenous retroviruses acting as IFN-a inducers has also been formulated, but most of these do not induce interferon alpha.

Apoptotic cells or necrotic cells could also cause IFN-a induction in AGS in the presence of PBMC or different cells of the glia. Two years ago we had observed a child who had an abnormal response to rifabutin, an antimicrobacterial agent. This child has produced very high levels of interferon-a in the blood after an abnormal response to this substance, with a pancytopenia. Probably, apoptotic or necrotic cells in this patient have induced interferon alpha. Concerning the lupus disease, (Vallin 1999) has induced interferon alpha in PBMC with apoptotic cells bound with antibodies of the patient’s serum.

Finally, one other hypothesis is that the accumulation of metabolites of DNA or RNA during a DNA repair disease could induce interferon alpha. However, we do not detect IFN-a in patients with a disease such as Cockayne syndrome.

-Role of interferon-alpha in AGS
The second major question concerning AGS is: Has interferon a pathogenic role in the disease? Interferon alpha injected for therapy of cancer or hepatitis induce an “interferon syndrome” associated with fever and nausea, aches and other signs. This syndrome of short duration is similar to the influenza syndrome. In the IFN treated patients interferon-a does not cross the blood brain barrier and rarely induces side effects in the CNS. In contrast, IFN-a is produced inside the CNS of children affected with AGS. These children produce high levels of interferon every day and we have estimated that, with a level of 100 IU per ml in the CSF, about 10-5 units per day are secreted during months or years. Interferon type I upregulates more than 200 cellular genes which have multiple biological effects; several genes including mitochondrial genes are downregulated (Der1998). Interferon-a also induces the presence of cellular structures like those shown by Peter Barthes this morning. Here on this slide, the same tubuloreticular inclusions are visible in a skin biopsy from a patient of Jean Aicardi and Françoise Goutières. These inclusions are located in the cytoplasm of the endothelial cells. These tubuloreticular inclusions were described at first in lupus erythematosus disease in the seventies and for a long time virologists and other pathologists believed that it was the virus of lupus. Steven Rich(1980) showed that, after he put interferon-a on white cells or on fibroblast cells, these inclusions appeared 2 or 3 days later. In addition, vasculitic lesions bearing similarities to the chilblains seen in Aicardi-Goutieres syndrome (Tolmie 1995; Stephenso 1997) and in Cree encephalitis (Black 1988) have been reported during treatment with interferon alpha (Bachmeyer1996; Creutzig1996; Campo-Voegeli 1998; Cid (1999). We can easily imagine that all the effects of IFN may strongly disturb the function and the structures of cellular components of the CNS. Convincing experiments performed (Akwa1998) ( Ian Campbell (1998-1999) suggests a possible causal relationship between abnormal intrathecal syntheses of this cytokine. He obtained transgenic mice that expressed alpha interferon specifically targeted in
astrocytes (inside the brain). He selected different strains of mice, of which some had a high expression of IFN and developed an early disease at 3 months of age, and others had a low expression of IFN and began to be ill at 10 or 12 months. These transgenic mice with astrocyte-specific chronic overproduction of IFN-a showed neuro-pathological features mimicking those found in Aicardi-Goutieres. The uniqueness of this model is the spread of the vascular inflammation with calcification of the basal ganglia, a leucodystrophy with presence also of the intracellular tubuloreticular inclusions, and an up-regulation of various genes including the gene of an adhesion molecule ICAM1. Other experiments show the deleterious effect of IFN-a on CNS: indeed, in neonatal infection by lymphocytic chronic meningitis virus (LCMV), the suppression of endogenous IFN by antibodies improves the animals (Riviere1977). These findings lead us to hypothesize that Aicardi-Goutieres syndrome may represent a primary genetic 'interferonopathy' (Crow 2001 unpublished data).

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