

# **Role of Viral Infections in Chronic Fatigue Syndrome**

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# Overview of Viruses and Chronic Fatigue Syndrome

- **Mesh heading search in the National Library of Medicine for years 1997-2007**
- **Mesh headings: fatigue syndrome, chronic and viruses**
- **Assessed only papers that compared viral infections in populations of CFS patients and healthy control subjects**
- **Major viral candidates:**
  - **HHV-6 (3 of 7 papers supportive)**
  - **HCMV (2 of 5 papers supportive)**
  - **EBV (2 of 4 papers supportive)**
  - **HHV-7 (1 of 4 papers supportive)**
  - **Enteroviruses (2 of 2 papers supportive) [tissues]**
  - **Parvoviruses (B19) (1 of 2 papers supportive)**

# Characteristics of the Human Herpesvirus Family

## *General Properties*

- Three subfamilies are recognized, designated alpha (HSV1, HSV2 , VZV), beta (CMV, HHV-6, HHV-7) and gamma (EBV, HHV-8).
- First infections usually occur in childhood, and seroprevalence is high (> 50% in adults) for all herpesviruses except HHV-8.
- In virtually all cases lifelong latent infections are established.
- Reactivations of the viruses from latency are common.
- Minority of people suffer clinical illness, sometimes severe, as a result of herpesvirus reactivations. Chronic, active infections are sometimes established.

# **Natural History of Primary HHV-6 Infections**

# Natural History of Primary HHV-6 Infection

## *Exanthem Subitum*

- First described by Zahorsky in 1910
- Characterized by high fever for several days
- Maculopapular rash occurs in 10% to 40% of cases
- HHV-6 shown to be causative agent in 1988
- >90% of children infected by two years of age

# Natural History of Primary HHV-6 Infection

## *Hematological Complications*

- **Significant decreases in total peripheral blood leukocytes, lymphocytes and neutrophils occur during acute HHV-6 infections**
- **Neutropenia, thrombocytopenia, leukopenia and lymphopenia and CD4+ T lymphopenia have been reported**

# **Natural History of Primary HHV-6 Infection**

## ***Central Nervous System Complications***

- **Febrile seizures occur in 13% to 56% of infants presenting to ER with acute HHV-6 infection**
- **Numerous cases of severe or fatal HHV-6 encephalitis have been reported in infants**

# Natural History of Primary HHV-6 Infection

## *Respiratory System Complications*

- Severe lower respiratory tract involvement occurs in 15% of children seeking medical treatment for acute HHV-6 infection
- Fatal pneumonitis in an 18 month old, immunocompetent child has been reported

# Natural History of Primary HHV-6 Infection

## *Summary: Clinical Manifestations*

- Bone marrow suppression
- CD4+ T lymphopenia
- Encephalitis
- Acute pneumonitis

# **A and B Variants of HHV-6**

# Epidemiology of HHV-6 Variant A

- Roseola (exanthem subitum) is HHV-6 variant B
- <2% HHV-6 variant A by age of two years
- 44% HHV-6 variant A as primary infections in Uganda
- Approximately 10% HHV-6 variant A by age of 12 years in USA
- 71% HHV-6 variant A in normal lung tissues in USA

# A and B Variants of Human Herpesvirus Six

## *Comparison of Biologic Properties of HHV-6A and HHV-6B*

- Overall, nucleotide sequence identity between HHV-6A and HHV-6B is 90%
- Nucleotide sequence divergence between HHV-6A and HHV-6B clusters in immediate early/regulatory genes
- HHV-6A is able to infect and completely replicate in more cell types than HHV-6B, including macrophages and NK cells
- HHV-6A but not HHV-6B induces high level production of TNF $\alpha$  in blood and bone marrow leukocytes

**Active HHV-6 Infections  
in Patients with Chronic  
Fatigue Syndrome and  
Relapsing-Remitting MS**

# Comparative Study of HHV-6 Infections in Patients with CFS and Relapsing-Remitting MS

## *Why compare multiple sclerosis and chronic fatigue syndrome?*

- Both are chronic, debilitating diseases
- Both have important fatigue components
- Both involve significant neurocognitive impairments
- Both may be triggered and/or perpetuated by a viral infection

# Comparative Study of HHV-6 Infections in Patients with CFS and Relapsing-Remitting MS

## *Characteristics of Study Populations*

### Relapsing-Remitting MS

- 28 patients with short duration (mean = 4 years) with relapsing remitting MS
- Blood sample obtained at time of clinical disease relapse
- Another blood sample was obtained after relapse had resolved (mean interval = 59 days)
- Blood samples assessed for active HHV-6 infection by rapid culture, antigenemia, and nested plasma PCR

# Comparative Study of HHV-6 Infections in Patients with CFS and Relapsing-Remitting MS

## *Characteristics of Study Populations*

### Chronic Fatigue Syndrome

- Blood samples were submitted for diagnostic testing from a single physician specializing in CFS patients
- 75 consecutive blood samples submitted between May and November, 2005
- Blood samples were assessed for active HHV-6 infection by rapid culture, antigenemia, and nested plasma PCR

# Comparative Study of HHV-6 Infections in Patients with CFS and Relapsing-Remitting MS

## *Characteristics of Study Populations*

### Healthy Control Subjects

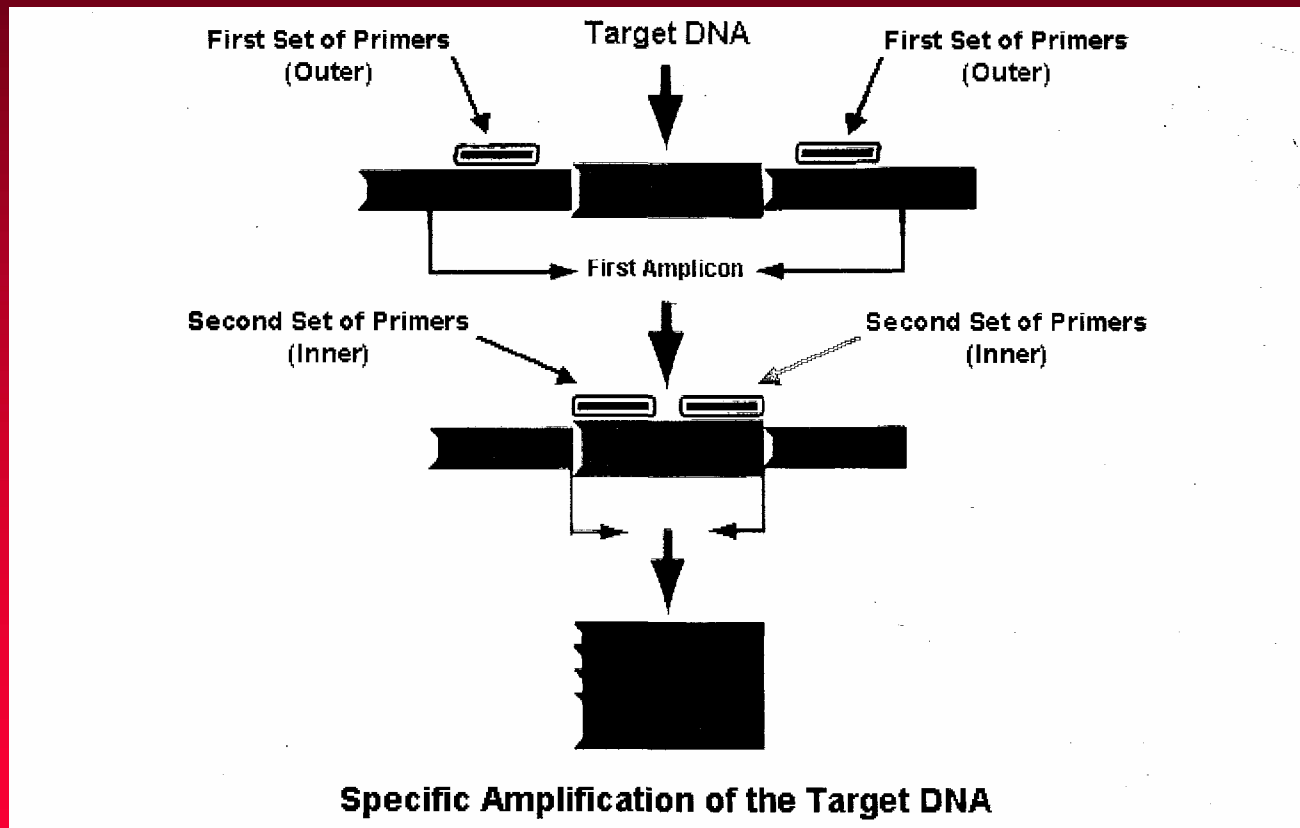
- Whole blood samples were obtained after thorough screening from normal blood donors at a private blood bank
- Serum samples were obtained from a commercial source (Analytical Biological Services Inc.; Wilmington, Delaware)

# Methods for the Diagnosis of Human Herpesvirus Six Infections

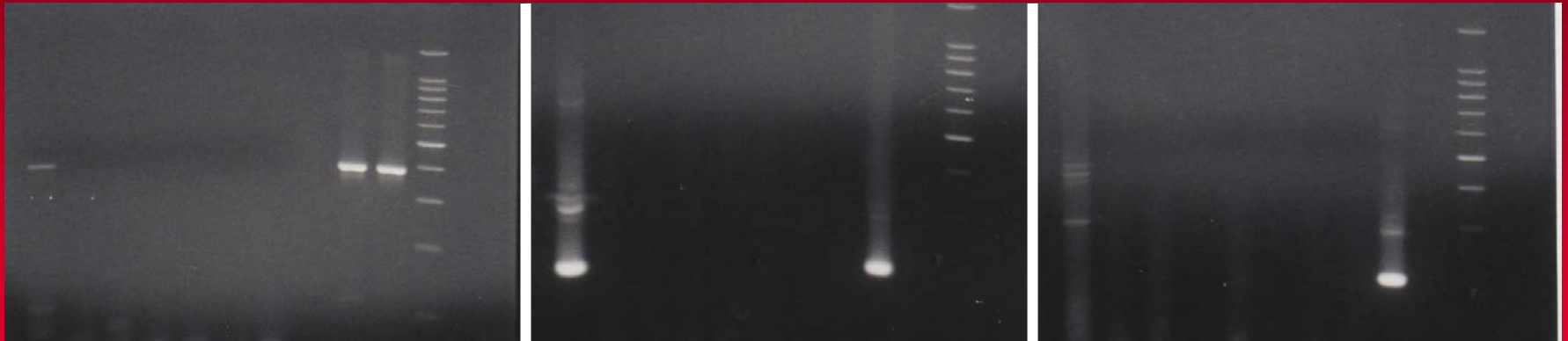
## *Diagnostic Methods Used By Wisconsin Viral Research Group*

- Nested HHV-6 Plasma PCR
- Immediate Early Protein Specific Antigenemia
- Rapid HHV-6 Culture

# Detection of HHV-6 DNA By Nested Polymerase Chain Reaction



# Detection of HHV-6 DNA By Nested Polymerase Chain Reaction

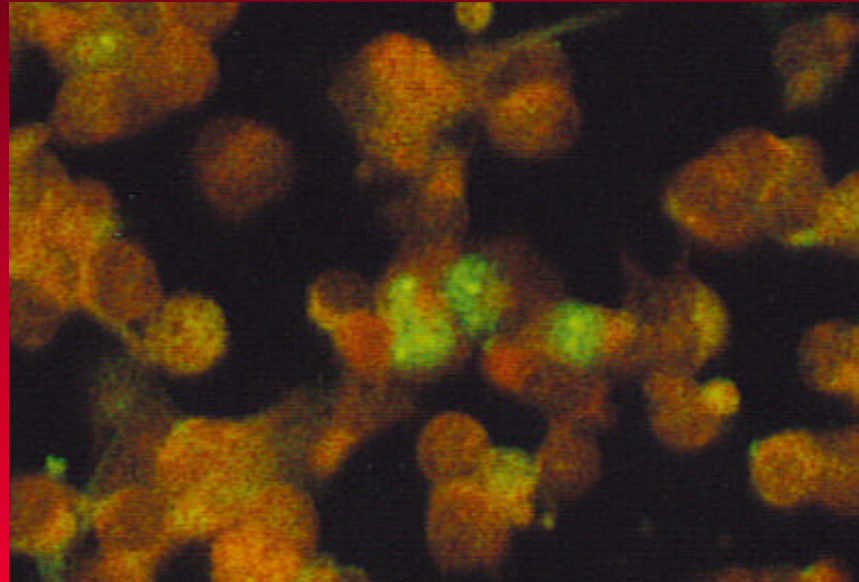


**First  
Round**

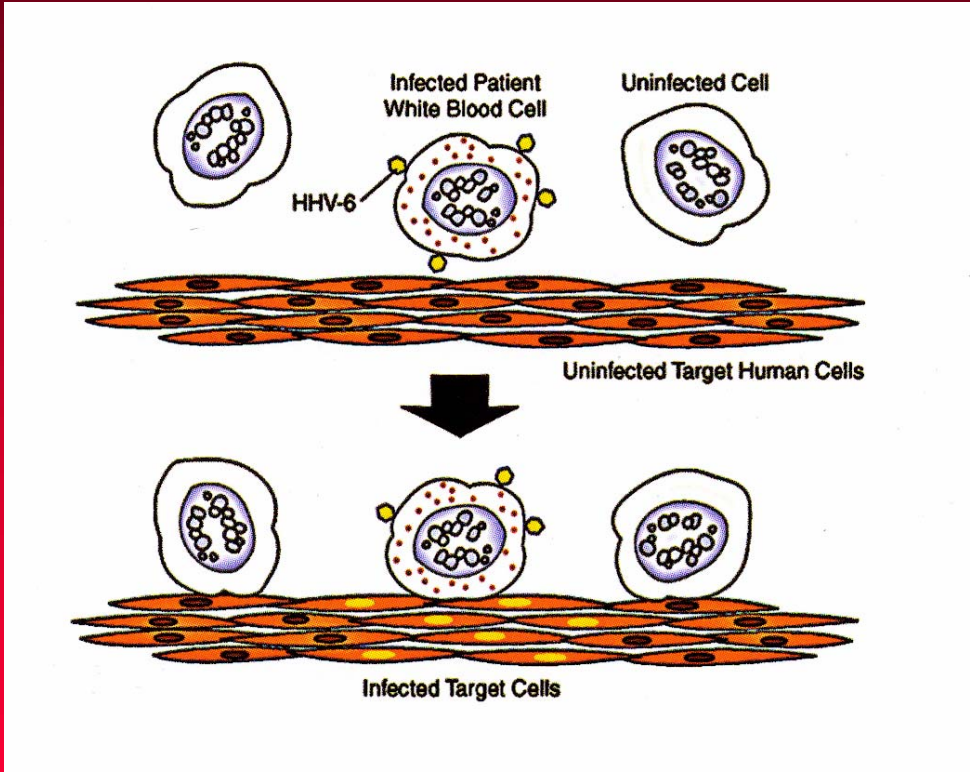
**Variant A  
Second Round**

**Variant B  
Second Round**

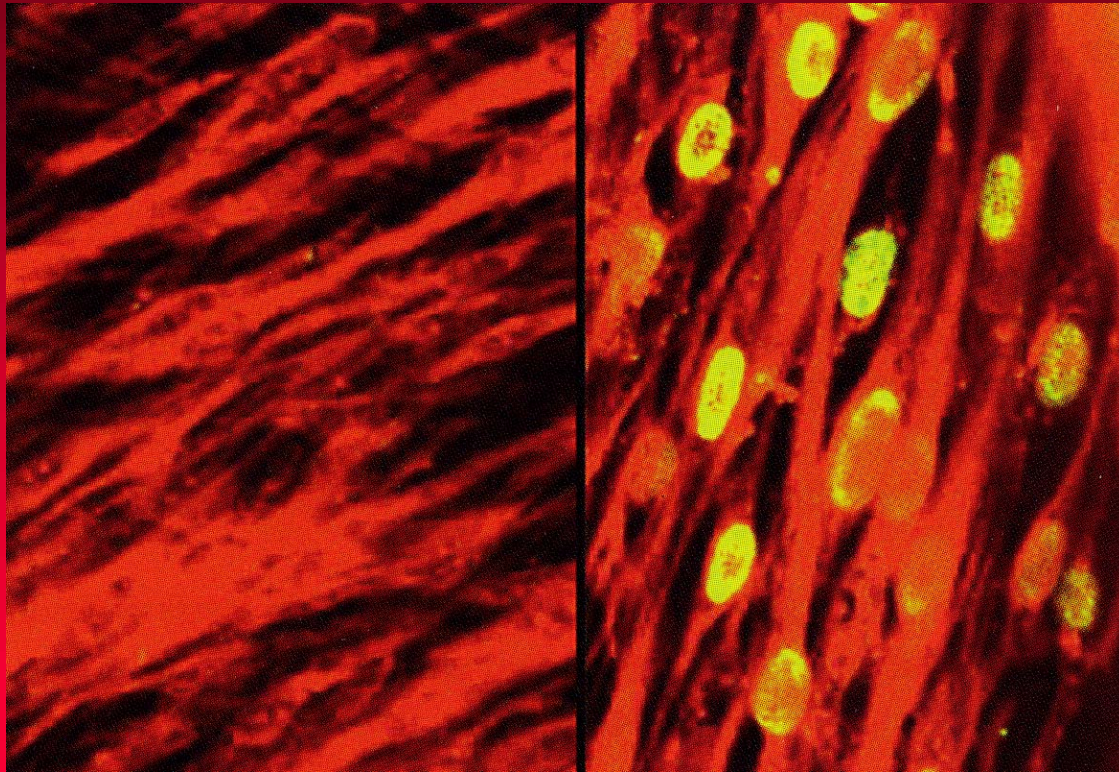
# Detection of HHV-6 Infections By Immediate Early Protein Antigenemia



# Detection of HHV-6 Infections By Immediate Early Protein Rapid Culture

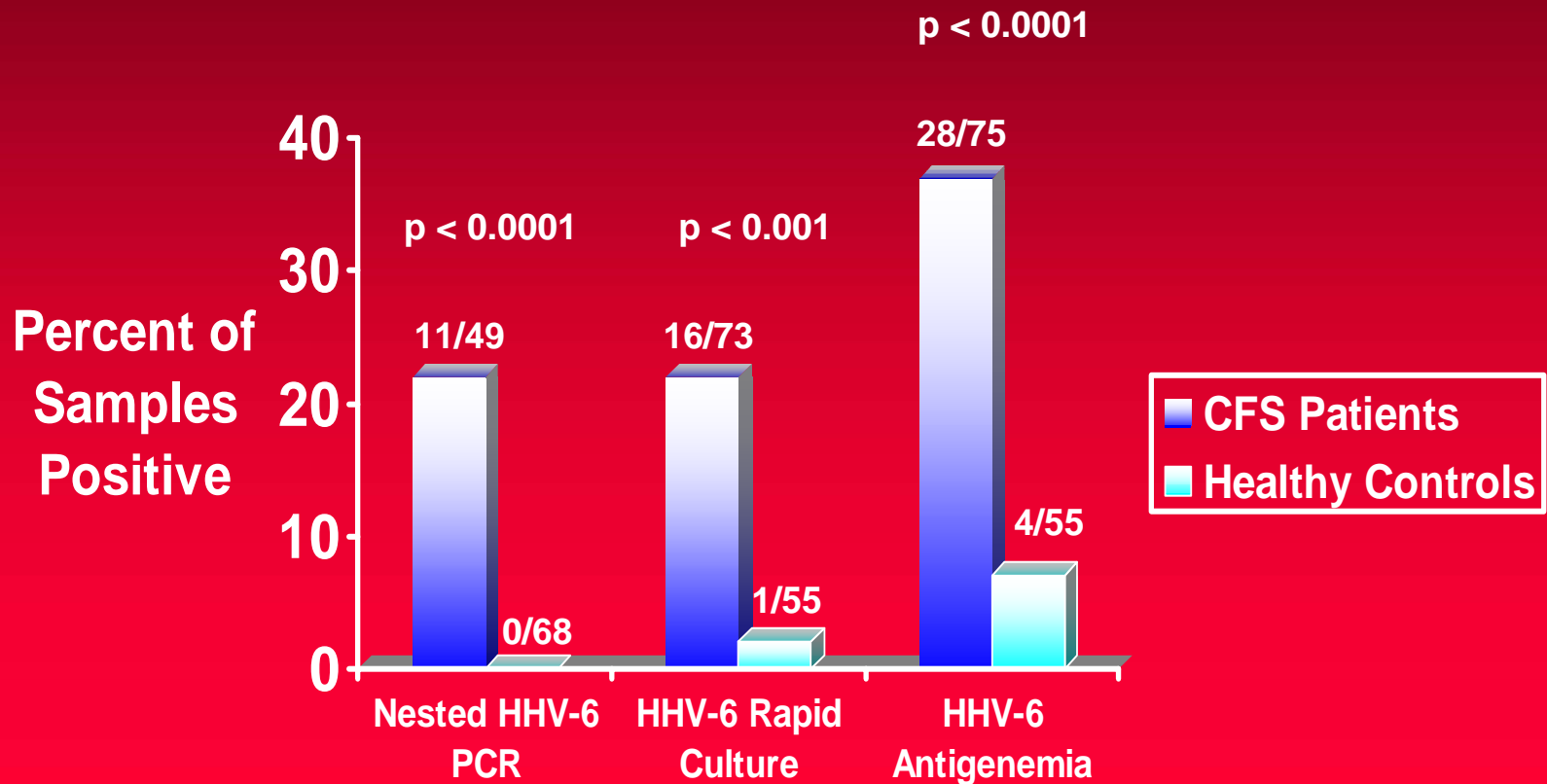


# Detection of HHV-6 Infections By Immediate Early Protein Rapid Culture



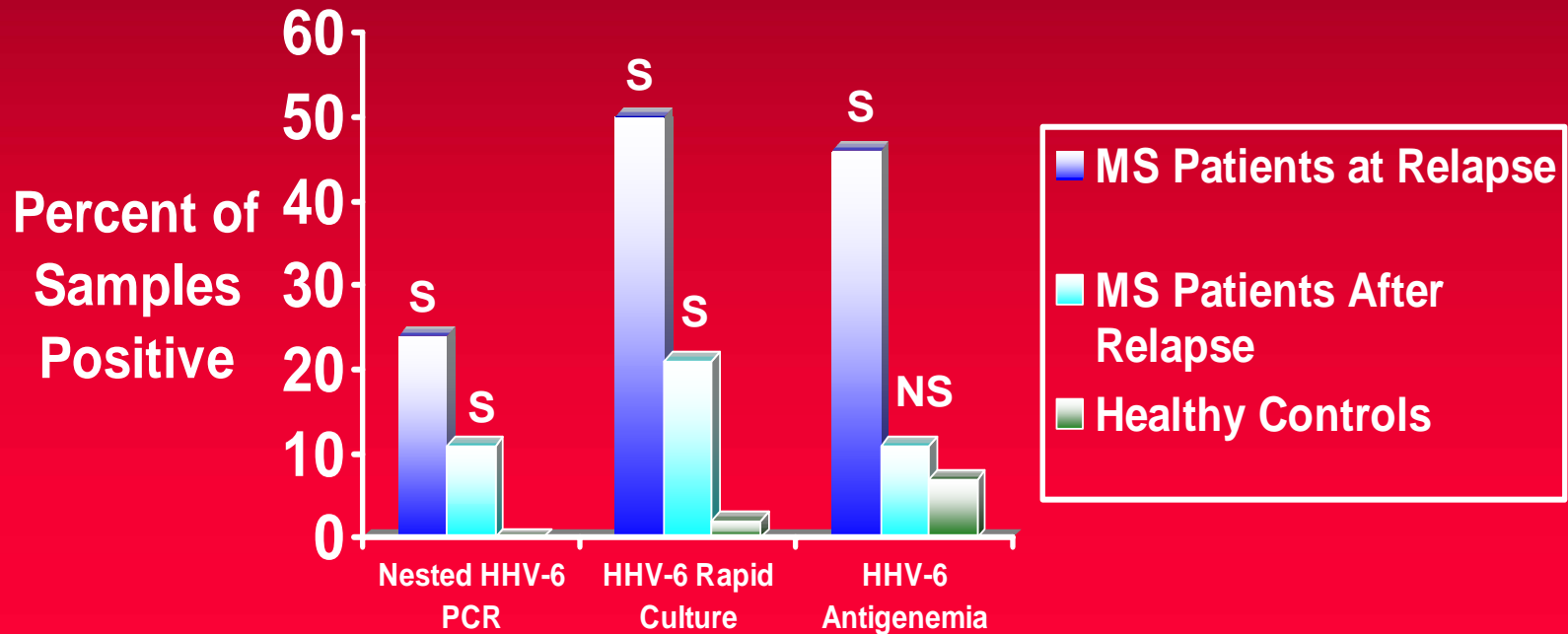
# Detection of HHV-6 Infections In Patients with CFS and Healthy Controls

## Comparison of Testing Methodologies Using Cross Sectional Blood Samples



# Detection of HHV-6 Infections In Patients with MS and Healthy Controls

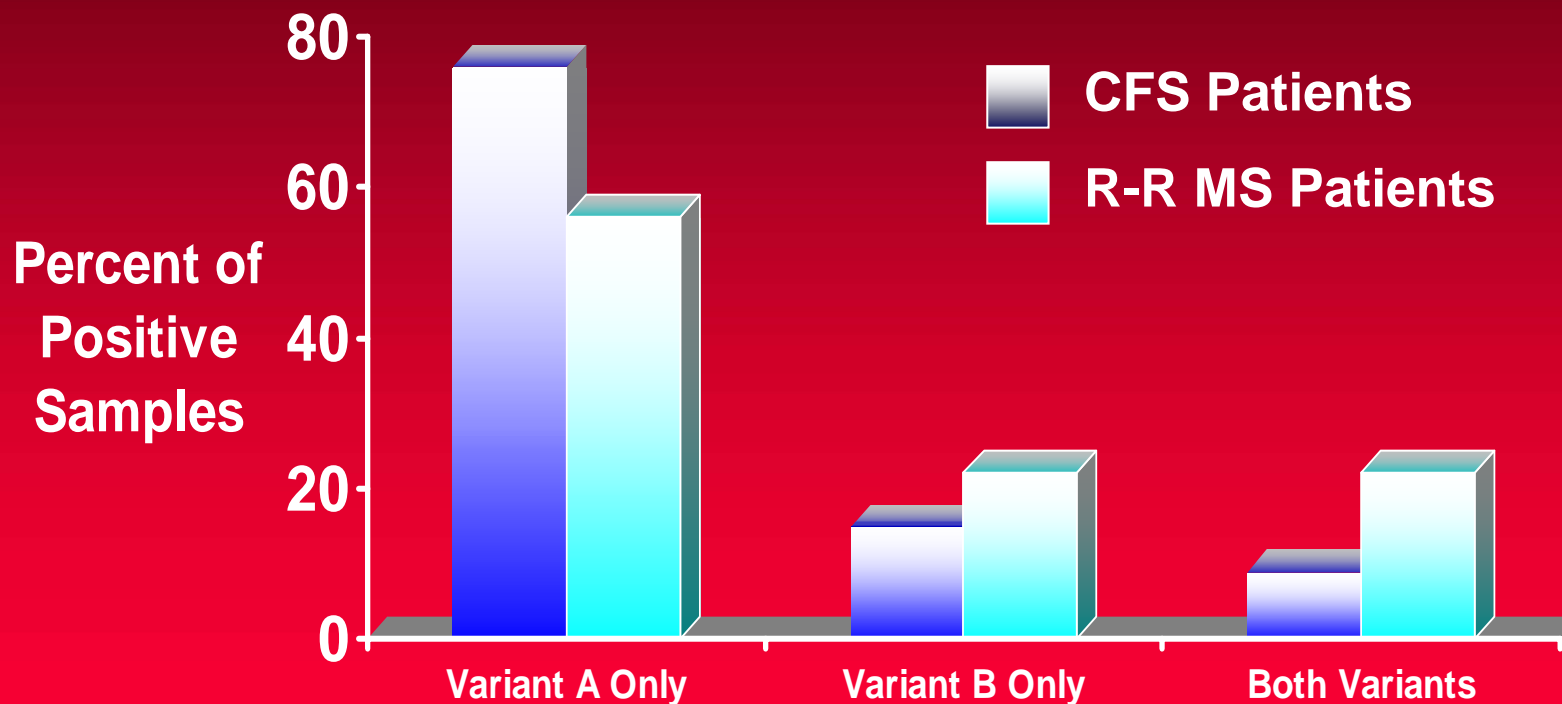
## Comparison of Testing Methodologies Using Cross Sectional Blood Samples



S: Significant difference compared to controls    NS: Not significantly different from controls

# Detection of HHV-6 DNA In Plasma Samples from CFS and MS Patients by Nested PCR

## *Determination of Viral Variant Involved*

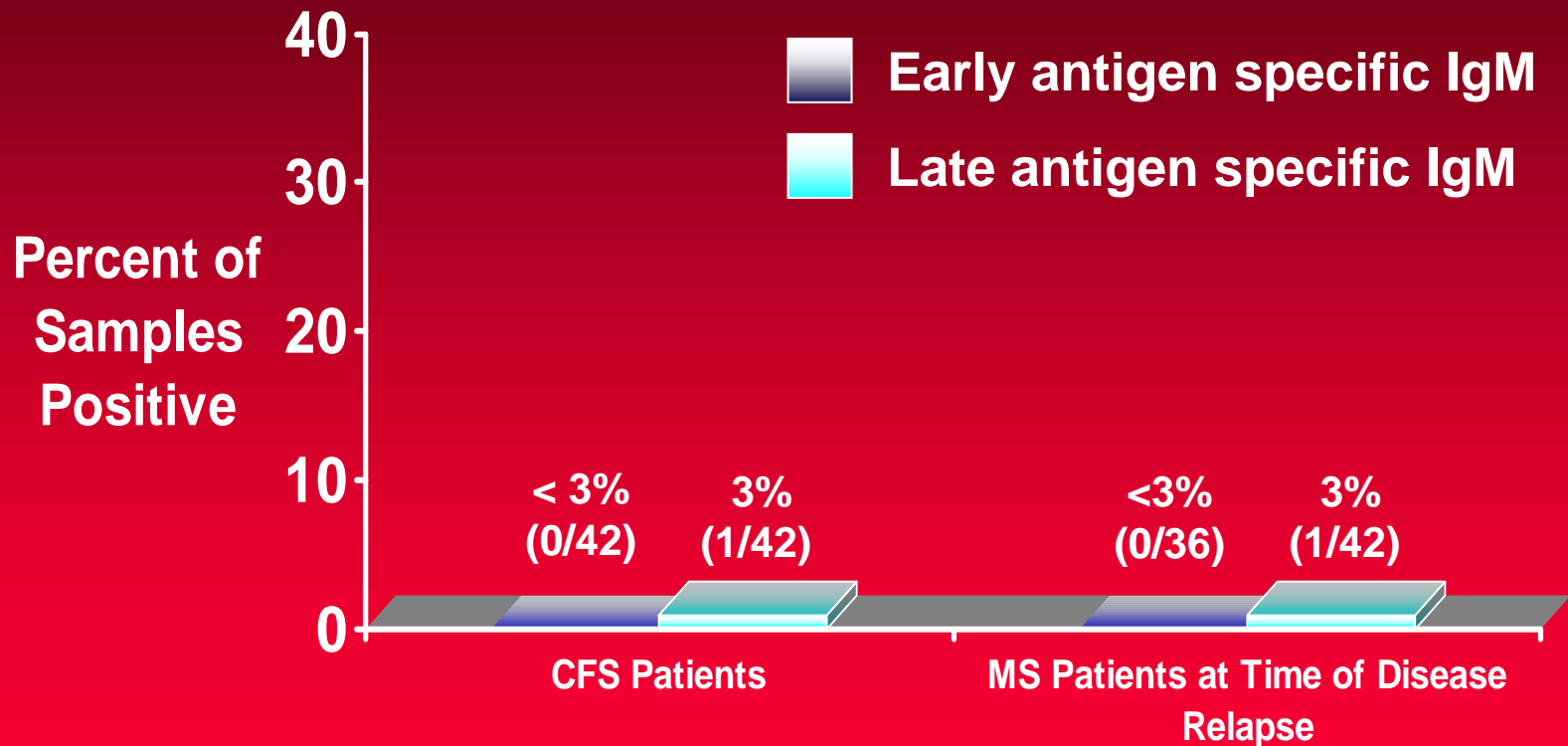


# Comparative Study of HHV-6 Infections in Patients with CFS and Relapsing-Remitting MS

## *Comparison of Diagnostic Technologies*

- **Nested Plasma PCR: Provides specific data with respect to viral variant but is not very sensitive**
- **Rapid culture and antigenemia: Sensitive but labor intensive and requires extended turn around time**
- **Is there a workable alternative technology for screening patients for active HHV-6 infections?**

# Detection of HHV-6 Infections by Early<sup>1</sup> and Late Antigen<sup>2</sup> Specific IgM Antibodies



1 Indirect immunofluorescence assay using ganciclovir blocked HHV-6A infected HSB-2 cells

2 Indirect immunofluorescence assay using HHV-6A infected HSB-2 cells

# Comparative Study of HHV-6 Infections in Patients with CFS and Relapsing-Remitting MS

## *Conclusions*

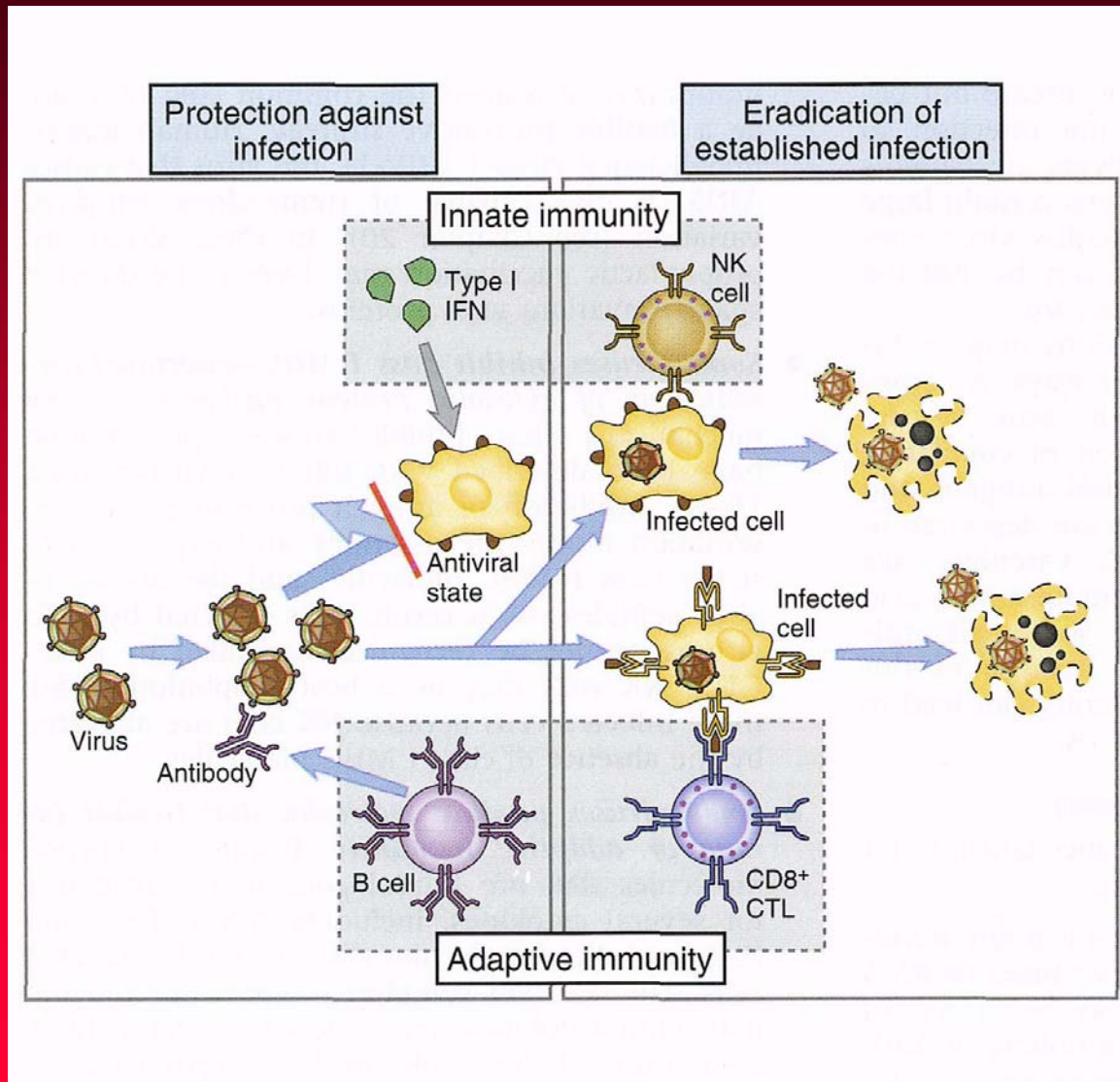
Both CFS patients and patients with early relapsing-remitting MS frequently have active HHV-6 infections in their blood leukocytes and tissues.

In the MS patients the active HHV-6 infections are significantly associated with clinical relapse of the patients' disease.

In both patient populations the variant involved in the infections is predominantly HHV-6A although HHV-6B infections do occur.

**Do patients with CFS  
have immunologic  
defects that make them  
vulnerable to viral  
infections?**

# Immune Responses to Viruses

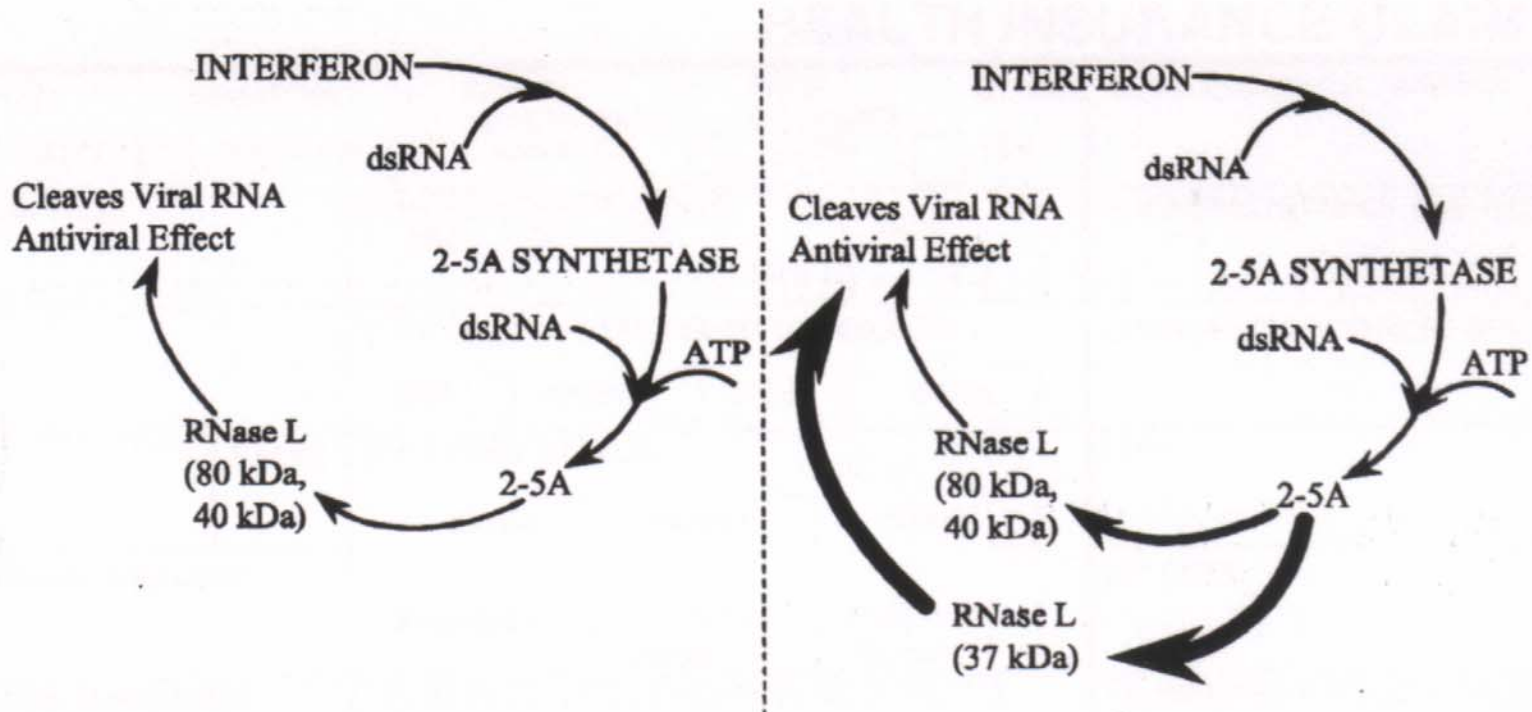


# Evidence for Dysregulation of Antiviral Pathway in Patients with CFS

- **Natural killer cells:**  
**Involved in viral and tumor cell surveillance**  
  
**Consistent evidence of impaired cytolytic activity in CFS patients**
- **Interferon response pathway**  
**i.e. the 2-5A/RNase L/STAT1 pathway**  
  
**Consistent evidence for degradation of RNase L and abnormally low STAT1 levels in patients with CFS**

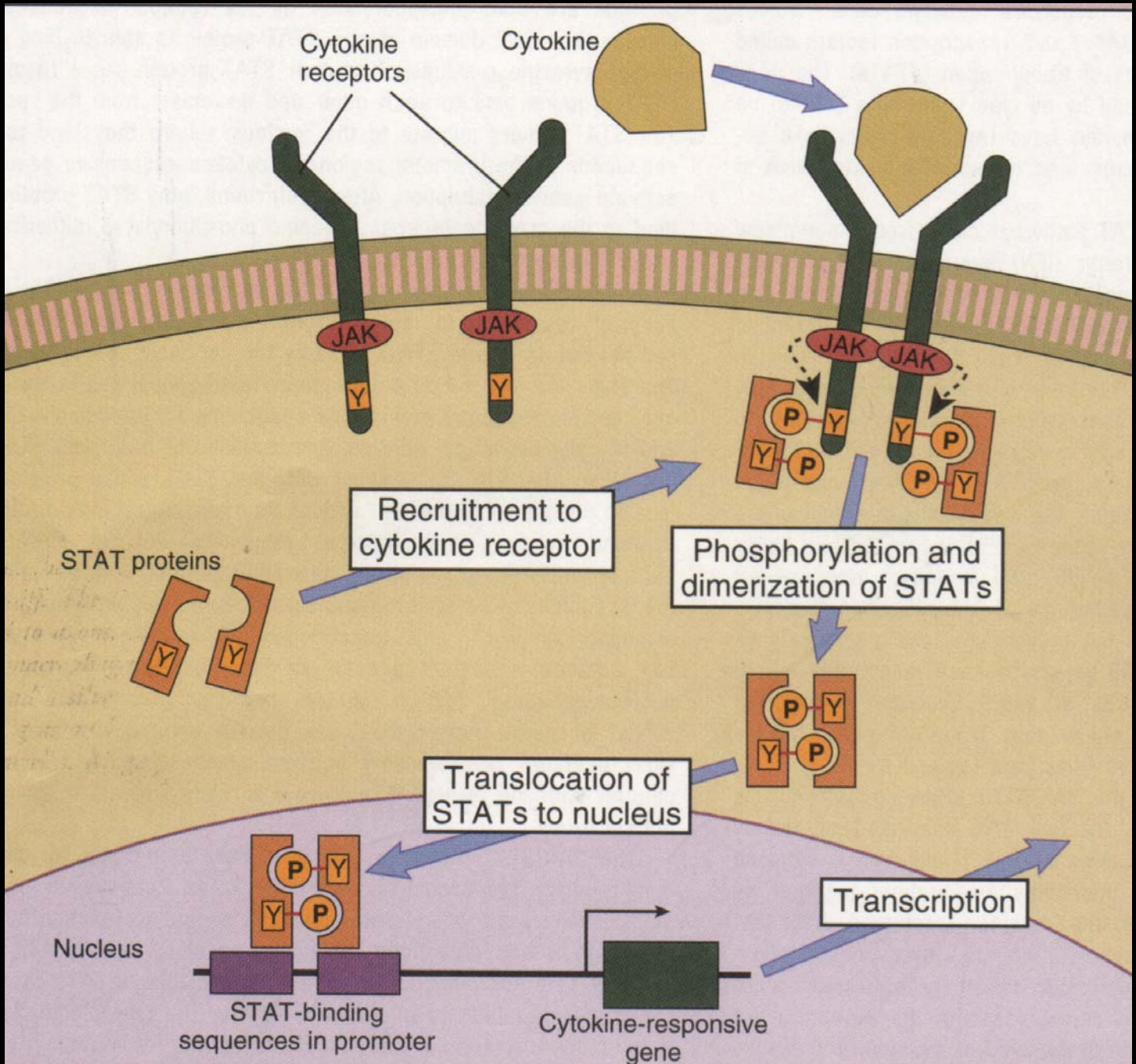
# Importance of STAT1 in CFS stems from recent observations suggesting that Type 1 interferon responses are abnormal in many patients with CFS

review by AL Komaroff; Amer J Med 108:169 (2000)



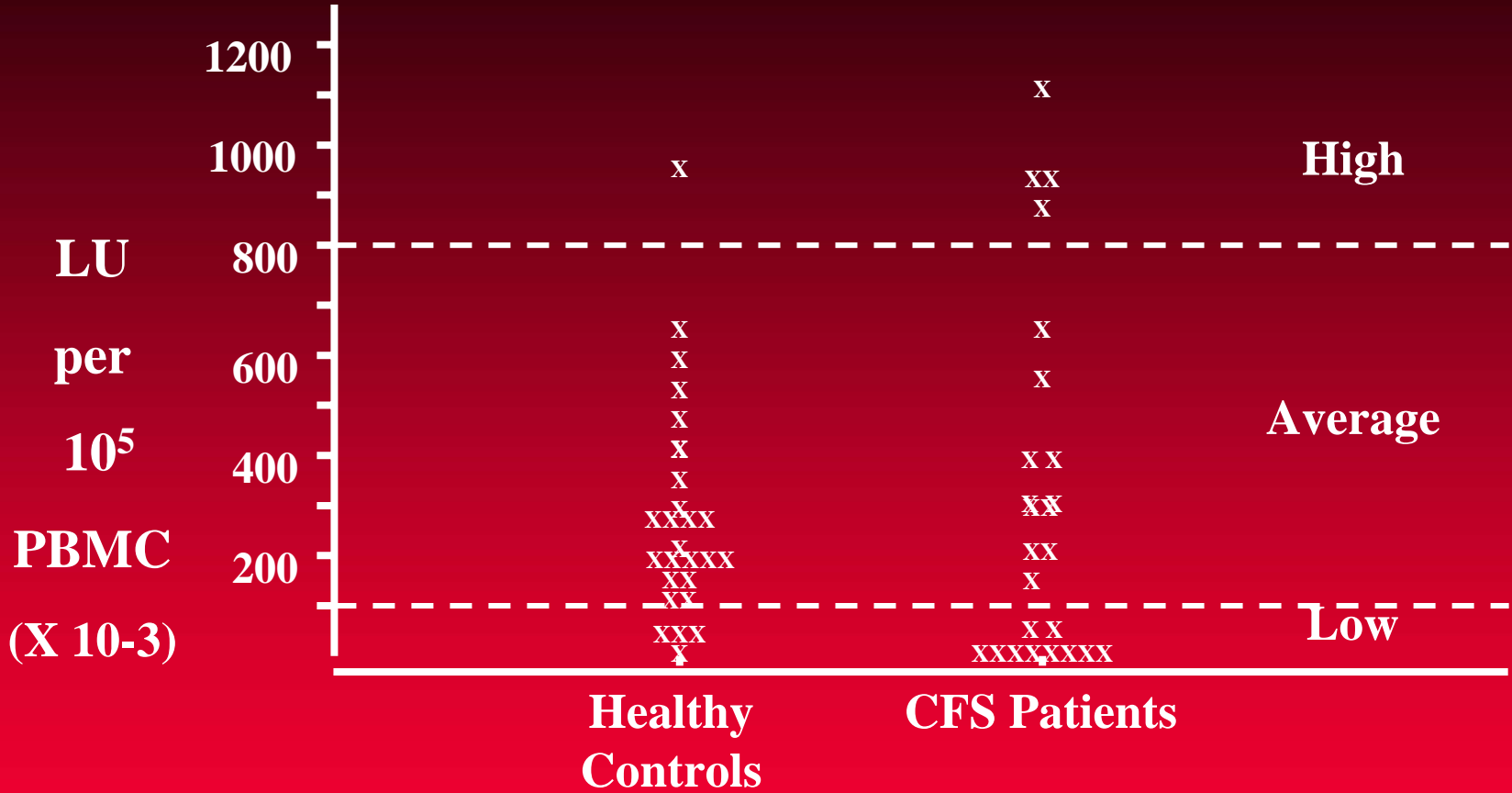
# STAT1

- **Centrally involved in the response of cells to**
  - Type 1 interferons (alpha and beta)**
  - Type 2 interferon (gamma)**
- **In both animals and humans, defects in STAT1 are associated with fatal infections by both viruses and bacteria.**



# **WVRG Study of STAT1 Protein Expression in CFS Patients**

- Blood samples from 25 CFS patients were studied from two physician based practices in Rochester, NY and Albuquerque, NM**
- Blood samples from 27 healthy control subjects were obtained from laboratory personnel or purchased from Analytical Biological Sciences Inc; Wilmington DE**



# Two-Sided Fisher's Exact Test Analysis of STAT1-91/84 Expression in Healthy Control Subjects and CFS Patients

	Healthy Controls	CFS Patients	Total
STAT1-91/84 Negative	1 (4%)	8 (32%)	9
STAT1-91/84 Positive	26 (96%)	17 (68%)	43
Total	27 (100%)	25 (100%)	52

**p = 0.0098; Very Significant**

# **WVRG Study of STAT1 Protein Expression in CFS Patients**

## **Conclusions**

- STAT1 is comprised of at least five antigenically related proteins with the two most fully studied being the STAT1-91 (alpha splicing variant) and STAT1-84 (beta splicing variant).**
- A subset (approximately 30%) of patients with CFS have a quantitative deficiency in the expression of STAT1-91/84 proteins.**
- This deficiency may predispose the patients to develop a variety of infections due to dysfunction of the type 1 and type 2 interferon systems.**

# Viral Infections In Patients with CFS

## Summary

- **Patients with CFS appear to have increased vulnerability to viral infections, especially from members of herpesvirus family.**
- **Vulnerability may be due to increase in viral burden as result of poor immune surveillance: defects in NKC function and IFN response pathway.**
- **Patients may suffer specific antiviral defects: STAT-1 deficiency resulting in failure of T-lymphocyte to response to gIFN, and HHV-6 infection and destruction of immune cells.**