Chronic Fatigue Syndrome and the pathophysiology of EBV infection: Trying to connect the dots to make sense

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Director, Institute for Behavioral Medicine Research, Professor, Molecular Virology, Immunology and Medical Genetics, Gilbert and Kathryn Chair in Medicine

First two papers published on Chronic Fatigue Syndrome

Jones, JF, et al., Evidence for active Epstein-Barr virus infection in patients with persistent, unexplained illness: Elevated anti-early antigen antibodies.
Ann Intern Med 1985; 102: 1-7.

Straus, SE et al., Persisting illness and fatigue in adults with evidence of Epstein-Barr virus infection.
Ann Intern Med 1985; 102: 7-16.

After 25 years of searching, we still do not have a consensus etiological agent. One virus, EBV, has been linked off and on over this period of time while HHV-6 has also appeared to be a player. More recently a retrovirus XMRV has generated some interest as a possible candidate. Whatever the agent(s) turns out to be, it is still necessary for researchers to account for the literature linking EBV/HHV-6 to CFS in my opinion.

Could the inconsistent results described in the literature be in part related to the lack of stress measures. Studies in the literature suggest that stress is a co-factor in CFS. In spite of that literature stress was not controlled for in most of those studies in spite of the fact that stress hormones such as cortisol can induce the reactivation of latent EBV.
Could there be more than one virus involved in the etiology of CFS, e.g. EBV and HHV-6 or just a retrovirus, e.g. XMRV with no connection with either herpesvirus or all of the above. These complicated interactions are hard to study but they could explain the difficulty of identifying an agent(s) in a heterogeneous group of patients clinically diagnosed with CFS.

- Because symptoms associated with CFS are also associated with an active virus infection, studies have focused on the hypothesis that a virus induces CFS.
- Several early studies showed that patients with these symptoms had significantly higher antibody titers to latent EBV. Holmes et al. [1], found higher EBV antibody titers in patients with symptoms compatible with CFS; antibody titers to other herpesviruses such as cytomegalovirus (CMV), Cameron, et al., herpes simplex virus (HSV), and measles virus have also been reported in CFS patients [2]. Buchwald et al. [3] and Hellinger et al. [4] also found a relationship between higher EBV antibody titers and CFS. Human herpesvirus 6 (HHV-6) has also been associated with CFS. For example, in an excellent review by Tony Komaroff in the Journal of Clinical Virology 2006, he presents data that are similar for HHV-6 to data obtained in recent studies involving XMRV. [5-9]
- To make things even more complicated, in two studies by Buchwald et al.,[10] and Koelle et al., [11], antibody titers and/or PCR were utilized to probe for several different viruses in CFS patients. No relation was found between antibody titers to any of 13 viruses tested, including latent herpesviruses, and no evidence for viral DNA in a variety of specimens was found. In summary, the association between EBV, HHV-6, other herpesviruses, and CFS has not produced a consensus on the etiology of CFS.

Epstein-Barr Virus Transactivates the Human Endogenous Retrovirus HERV-K18 that Encodes a Superantigen

From the Huber, Thorley-Lawson Laboratories

Immunity

15: 579-589, 2001

Brigitte Huber and colleagues have shown that the latent EBV encoded genes, LMP-1 and LMP-2A, can transactivate a human endogenous retrovirus HERV-K18 in HERV-K18 infected human B-lymphocytes. The interaction is with the envelope protein of HERV-K18, which encodes for a super antigen that can stimulate T-lymphocytes to produce significant levels of cytokines. These data support the possibility that EBV and an endogenous retrovirus could interact with clinical consequences. A super antigen is a class of antigens, which cause nonspecific activation of T-cells that results in polyclonal T-cell activation.

1. Holmes, G.P., et al., A cluster of patients with a chronic mononucleosis-like syndrome: is Epstein-Barr Virus the cause? *JAMA*, 1987. 257: p. 2297-30
2. Cameron, B., et al., Serological and virological investigation of the role of the herpesviruses EBV, CMV and HHV-6 in post-infective fatigue syndrome. *Journal of Medical Virology*, 2010. 82(10): p. 1684-8.
3. Buckwald, D., et al., Frequency of chronic active Epstein-Barr Virus infection in a general medical practice. *JAMA*, 1987. 257: p. 2303-7
4. Hellinger, W.C., et al., Chronic fatigue syndrome and the diagnostic utility of antibody to Epstein-Barr Virus early antigen. *JAMA*, 1988. 260: p. 971-3
5. Krueger, G.R., et al., Clinical correlates of infection with human herpesvirus-6. *In Vivo*, 1994. 8: p. 457-85.
6. Sairenji, T., et al., Antibody responses to Epstein-Barr Virus, human herpesvirus-6 and human herpesvirus 7 in patients with chronic fatigue syndrome. *Intervirology*, 1995. 38: p. 269-73.
7. Patnalk, M., et al., Prevalence of IgM antibodies to human herpesvirus 6 early antigen (p41/38) in patients with chronic fatigue syndrome. *J Infect Dis*., 1995. 172: p. 1364-7.
8. DiLuca, D., et al., Human herpesvirus 6 and human herpesvirus 7 in chronic fatigue syndrome., *J Clin. Microbiol*., 1995. 33: p. 1660-1.
9. Komaroff, A., Is Human Herpesvirus-6 a trigger for Chronic Fatigue Syndrome?, *Journal of Clinical Virology*, 2006. 37: Suppl: S39-46.
10. Buckwald, D., et al., Viral serologies in patients with chronic fatigue and Chronic Fatigue Syndrome., *J. Med. Virol*., 1996. 50: p. 25-30.
11. Ader, R., et al., Pychoneuroimmunology. Second ed. 1991, San Diego, CA: Academic Press, Inc.

“…social relationships, or the relative lack thereof, constitute a major risk factor for health - - rivaling the effects of well-established health risk factors such as cigarette smoking blood pressure, blood lipids, obesity, and physical activity.”

House, J.S., Landis, K.R., & Umberson, D.

“Social relationships and health.”

Science, 1988, 241:540-545

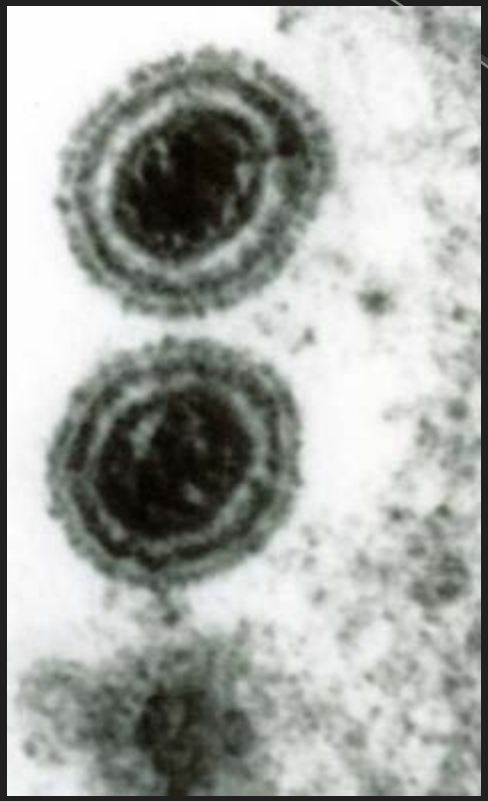
Hormone	Examples of Immune Cells with Receptors
Gluccocorticoids	T and B-lymphocytes, neutrophils, monocytes/macrophages
Substance P	T and B-lymphocytes, eosinophils, mast cells, monocytes/macrophages
Neuropeptide Y	T-lymphocytes, monocytes/macrophages
Corticotropin Releasing Hormone	T and B-lymphocytes, mast cells, monocytes/macrophages
Prolactin	T and B-lymphocytes, granulocytes, precursor cells, monocytes/macrophages
Growth Hormone	T and B-lymphocytes, monocytes/macrophages
Catecholamines (epinephrine/norepinephrine)	T and B-lymphocytes, neutrophils, NK cells, monocytes/macrophages
Serotonin	T and B-lymphocytes, NK cells, monocytes/macrophages

Glaser and Kiecolt-Glaser, *Nature Reviews Immunology*, 2005

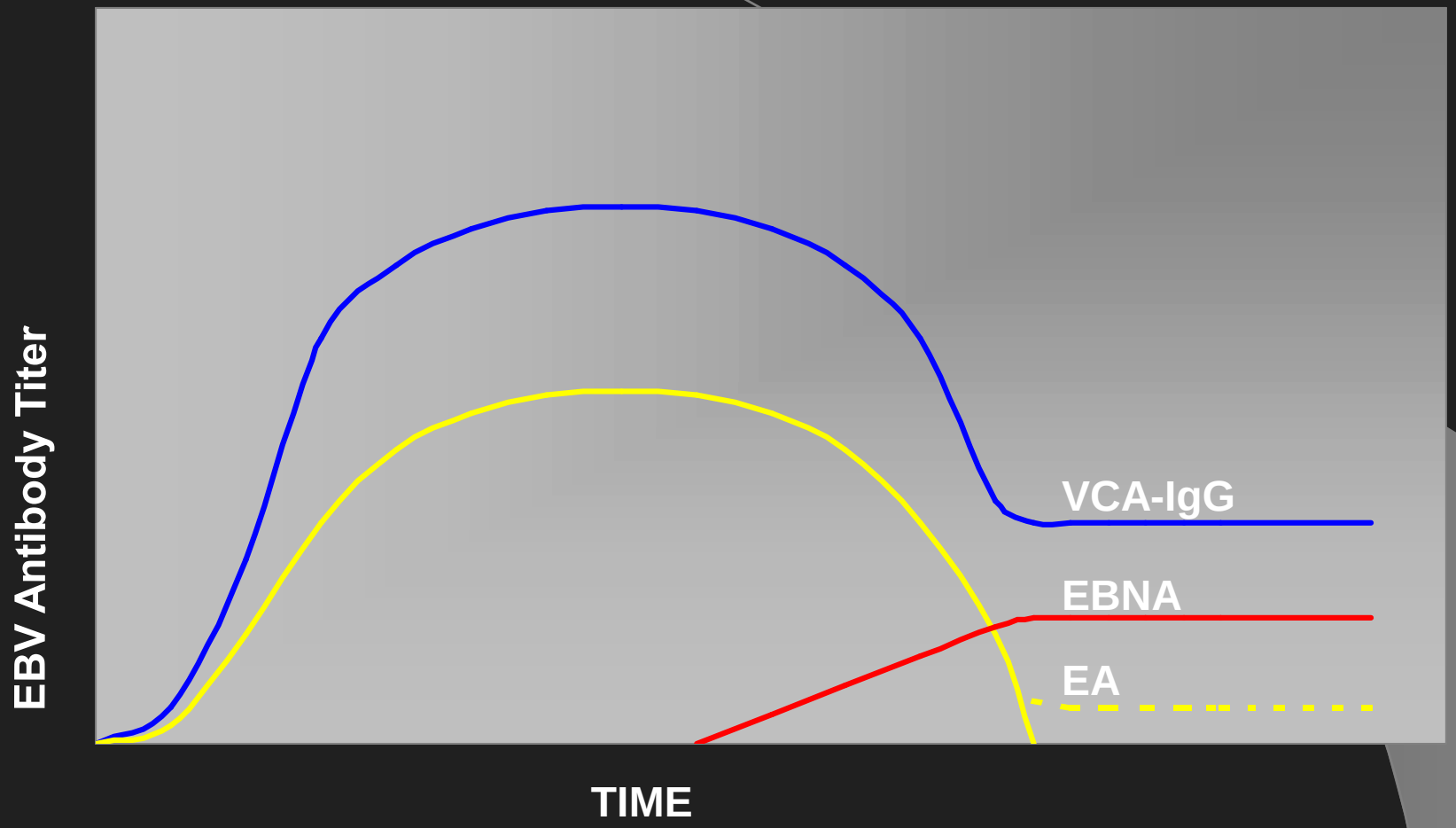
MEASURING STRESS--ISSUES

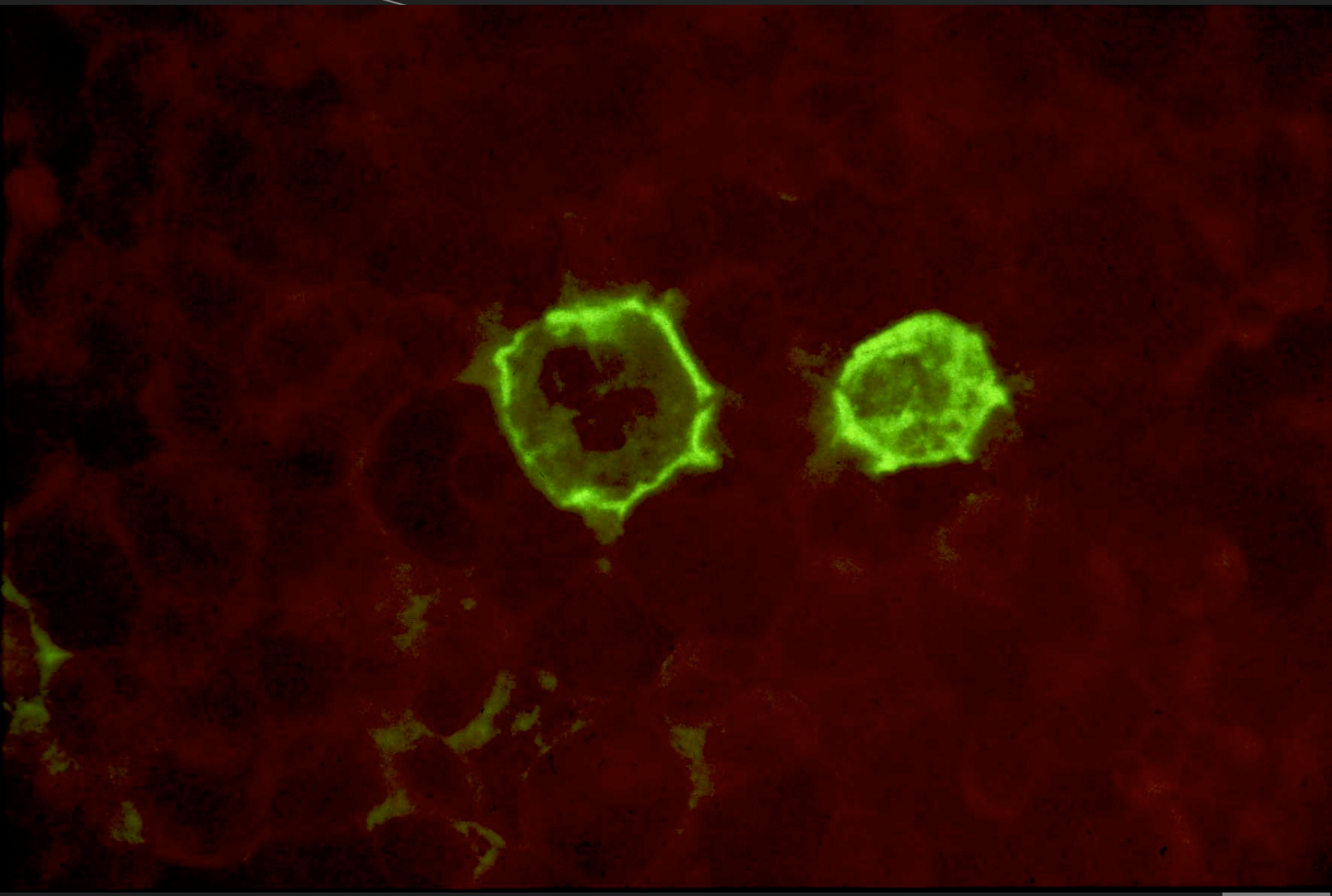
- One definition for stress is life events or environmental demands that tax or exceed the adaptive capacity of a person, that results in psychological and biological changes that may place persons at risk for disease
- Stress responses are influenced by
  - Characteristics of the stress, e.g. acute or chronic, predictability, controllability
  - Biological factors (age, gender, genetics)
  - Prior history with stressor, early life experiences, etc.

Electron Micrograph Typical Herpesvirus Particle



100 µm in diameter


EBV Antibody Titer
VCA-IgG
EBNA
EA
TIME

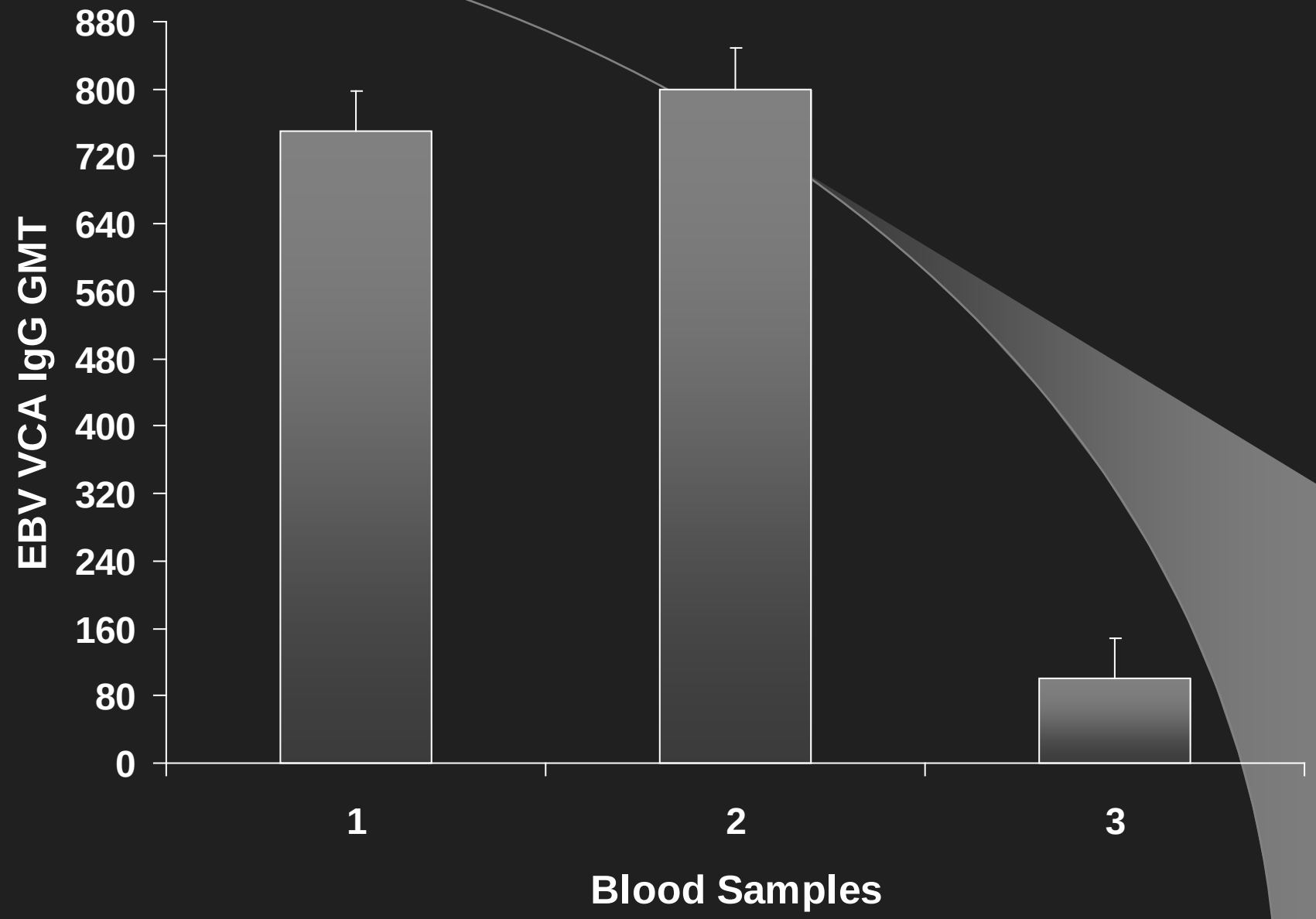


Medical Student Academic Stress Studies

- First and second year students (pre-clinical)
- Exams grouped in 3-day blocks:
  Wednesday (Exams), Thursday (Off), Friday (Exams)
- Blood samples drawn at noon on Fridays
- Students report greater stress/distress during exams than during “baseline” periods without exams

BSI Scale	Baseline	Examinations
Depression*	55.97	59.72
Anxiety***	53.39	59.72
General Severity Index***	56.75	61.17
Positive Symptom Distress Index***	53.36	60.39

*$p<0.01$ ***$p<0.0001$



Kiecolt-Glaser et al, Psychosomatic Medicine, 1984.

Means (±SEM) EBV VCA Antibody Titers

Sample	EBV VCA Titers
Baseline 1 (September)	68.18 (25.05)
Examinations (October)	115.04 (10.86)
Baseline 2 (January)	80.08 (15.86)
Examinations (February)	386.36 (101.30)
Baseline 3 (April)	84.85 (22.76)
Examinations (May)	141.03 (46.34)

N = 34

P = <.0001

Glaser, R., Rice, J., Sheridan, J., Fertel, R., Stout, J., Speicher, C., Pinsky, D., Kotur, M., Post, A., Beck, M., Kiecolt-Glaser, J. - *Brain, Behavior, and Immunity*, 1987.

The Effect of Examination Stress on the Memory T-Cell Response to Purified EBV Polypeptides[a]

	Baseline		Examination	
Polypeptide	CPM[1]	SI (± SEM)	CPM[2]	SI (± SEM)
gp250**	12734.02	1.54 (0.11)	7986.82	1.24 (0.09)
gp125	13478.60	1.63 (0.13)	7587.79	1.44 (0.13)
p160*	11792.41	1.42 (0.10)	9200.99	1.20 (0.11)
p85*	9771.88	1.15 (0.10)	6448.76	0.96 (0.08)
p52/50**	10113.31	1.21 (0.12)	6184.63	0.92 (0.11)
p17*	11440.76	1.39 (0.15)	8593.03	1.14 (0.14)

[a] As measured by proliferation of PBLs in the presence of 5 µg of each polypeptide. All cultures were incubated 112 hours, with a pulse of 1 µCi H^3 TdR for 8 hours.

* $p < .05$ ** $p < .01$

Glaser, Pearson, G.R., Bonneau, R.H., Esterling, B.A., Atkinson, C., and Kiecolt-Glaser, J.K , *Health Psychology*, 1993.

HEALTH DATA

"He's the typical American mouse—likes a drink before dinner, smokes a little, watches TV . . ."

Drugs (prescription / nonprescription)

Acute and chronic health problems

Change in sleep patterns

Weight change

Nutritional assays

Alcohol intake

Cigarette smoking

Caffeine intake

Epstein-Barr Virus Specific Salivary Antibodies as Related to Stress Caused by Examinations

O. Sarid, O. Anson, A. Yaari, M. Margalith

Journal of Medical Virology, June 2002, 64 (2): 149-156

A Novel Link Between Stress and Human Cytomagalovirus (HCMV) Infection: Sympathetic Hyperactivity Stimulates HCMV Activation
Prösch et. al., Virology, 2000, 272 (2): 357-365

Incidence of Epstein-Barr Virus in Astronaut Saliva During Space flight
Payne et a., Aviation, Space, and Environmental Med, 1999, 70(12):1211-1213

Epstein-Barr Virus Reactivation Associated with Diminished Cell-Mediated Immunity in Antarctic Expeditioners
Mehta et al., J Med Virol, 2000, 61:235-240

Stress-Induced Reactivation of Epstein-Barr Virus in Astronauts
Stowe et al., NeuroImmunoModulation, 2000, 8:51-58

Reactivation and Shedding of Cytomegalovirus in Astronauts during Spaceflight
Mehta et al., J Infect Dis, 2000, 182(6):1761-1764

Stress-Induced Subclinical Reactivation of Varicella Zoster Virus in Astronauts
Mehta et al., J Medical Virol 72:174-179, 2004.

Herpesvirus reactivation and socioeconomic position: a community-based study
Stowe et al., J Epidemiol Community Health, 64: 666-671, 2010.

Pitts, F.N., Allen, R.E., and Allen, A.D. In: Depressive Disorders and Immunity, American Psychiatric Press, Inc., Washington D.C., 1989.

Clinical Status and EBV Antibodies

	Major Depression			
	Severely Depressed	Depressed	Total	Controls
Antibodies to	(n = 71)	(n = 31)	(n = 102)	(n = 38)
VCA (IgG)	664 ± 665	437 ± 505	595 ± 622	325 ± 4

NOTE: VCA = viral capsid antigen; EA = early antigen; EBNA = Epstein-Barr nuclear antigen. Values are reciprocals of titers (Means ± SD).

[a] = Not detected for depressed patients or controls. SD = 4 for total patients with major depression.

Social Stress and the Reactivation of Latent Herpes Simplex Virus Type 1

David A. Padgett, John F. Sheridan, Julianne Dorne,
Gary G. Berntson, Jessica Candelora, Ronald Glaser

Proceedings of the National Academy of Science
(1998 Jun 9) 95: 7231-5

Influence of Restraint Stress on Ocular HSV-1 Reactivation

Treatment Group	Experiment 1	Experiment 2	Total
Control	0/16	0/10	0/26 (0%)
UV (250 mJ/cm^2)	7/16	5/10	12/26 (46.2%)
Restraint	1/15	1/10	2/25 (8%)
Restraint + UV	6/15	7/15	13/30 (43.3%)

Influence of Social Reorganization Stress on Ocular HSV-1 Reactivation

Treatment Group	Experiment 1	Experiment 2	Experiment 3	Total
Control	N/D	0/7	0/8	0/15 (0%)
UV (250 mJ/cm^2)	N/D	4/8	3/8	7/16 (43.7%)
SRO	6/17	5/10	4/10	15/37 (40.5%)
SRO +UV	9/18	6/9	6/10	21/37 (56.7%)

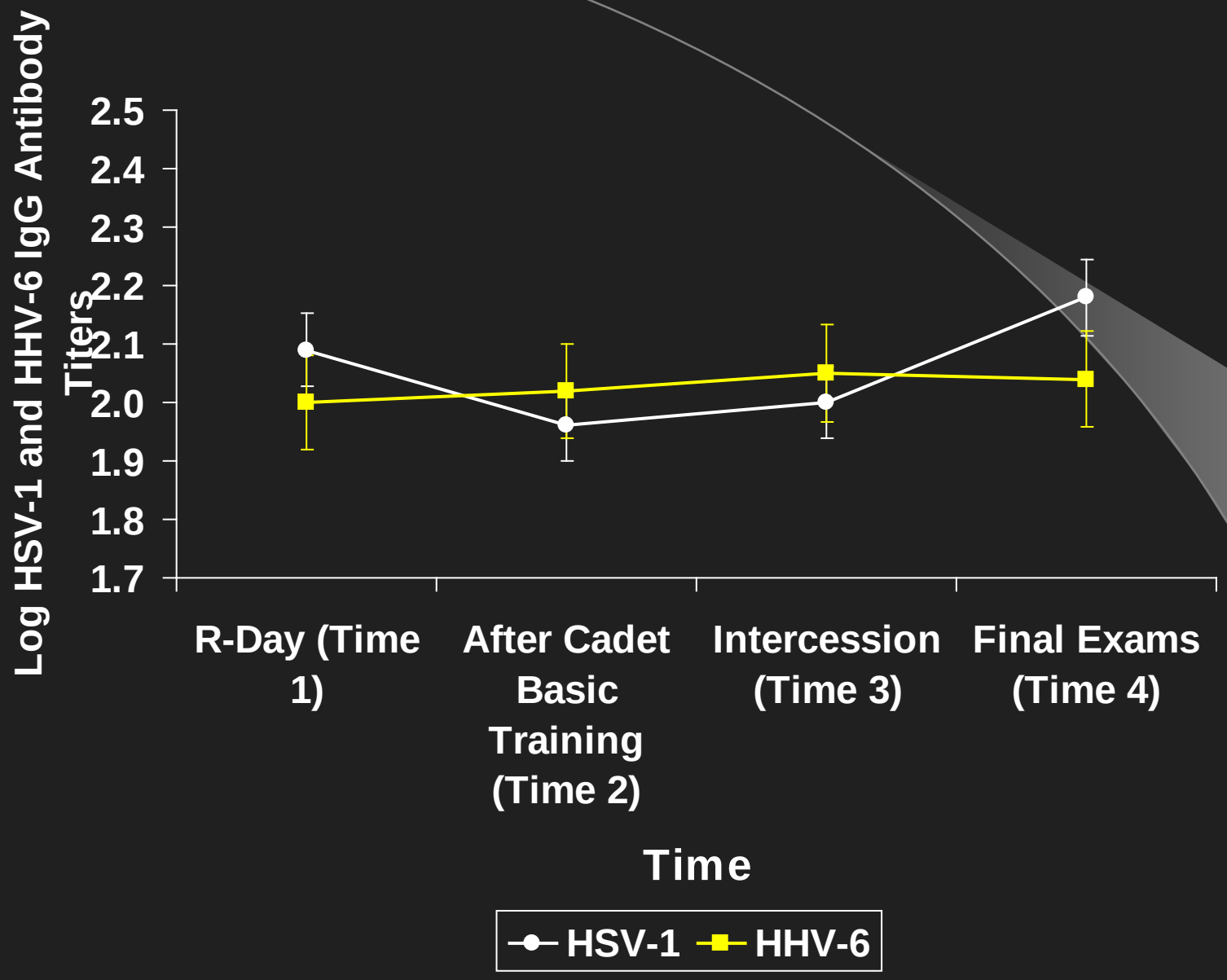
"He's the alpha gopher—the other guys are just gofers."

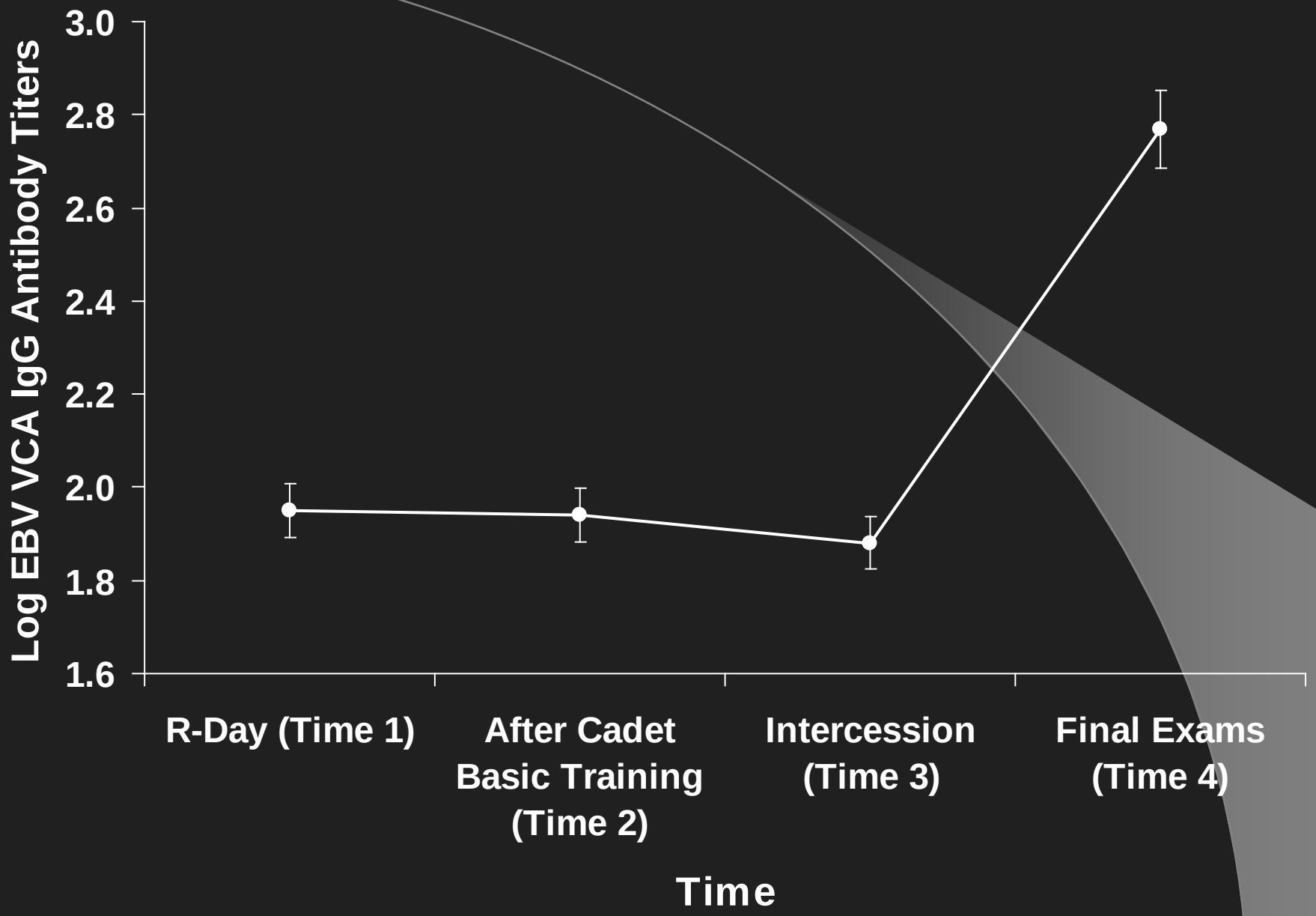
The Differential Impact of Training Stress and Final Examination Stress on Herpesvirus Latency at the United States Military Academy West Point

The study was supported by West Point and the Grant Foundation

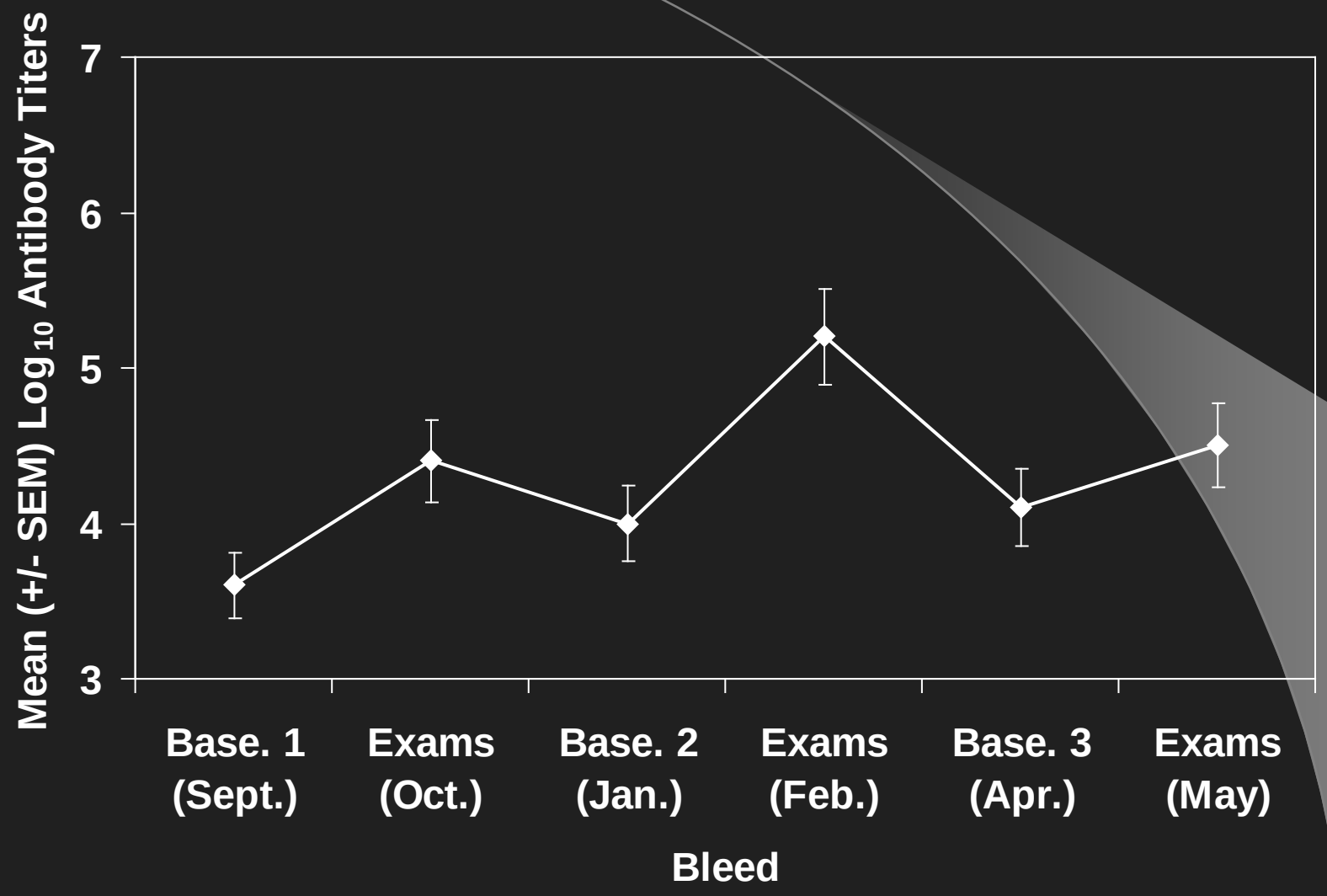
Ronald Glaser, Stanford Friedman, Joshua Smyth, Robert Ader, Polly Bijur, Philip Brunell, Nicholas Cohen, Leonard Krilov, Stephan Lifrak, Arthur Stone, Patrick Toffler

Brain, Behavior, & Immunity **13**: 240-251, 1999

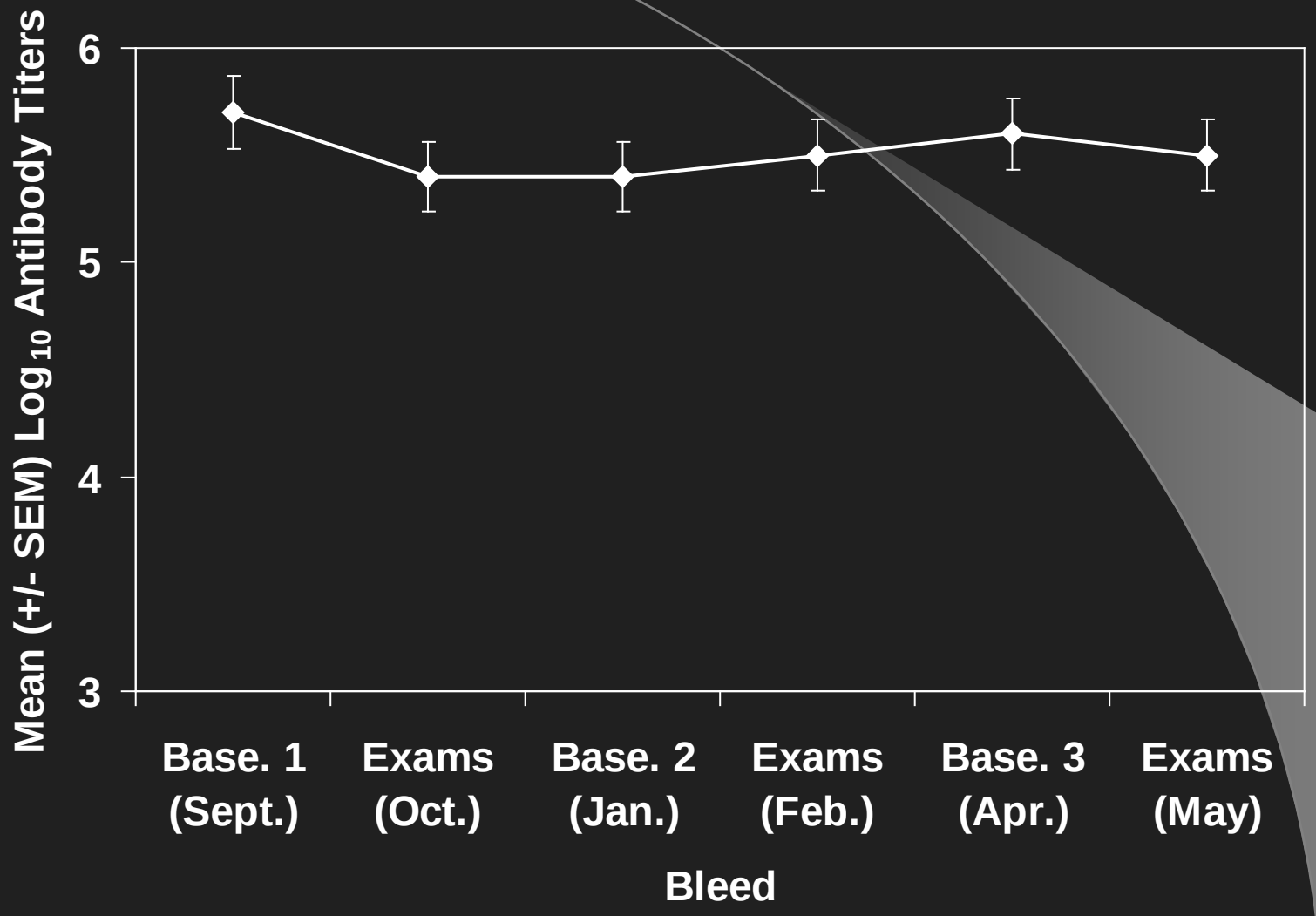

Log HSV-1 and HHV-6 IgG Antibody Titers
2.5
2.4
2.3
2.2
2.1
2.0
1.9
1.8
1.7
R-Day (Time 1)
After Cadet Basic Training (Time 2)
Intercession (Time 3)
Final Exams (Time 4)
Time
HSV-1
HHV-6


Log EBV VCA IgG Antibody Titers
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
R-Day (Time 1)
After Cadet Basic Training (Time 2)
Intercession (Time 3)
Final Exams (Time 4)
Time

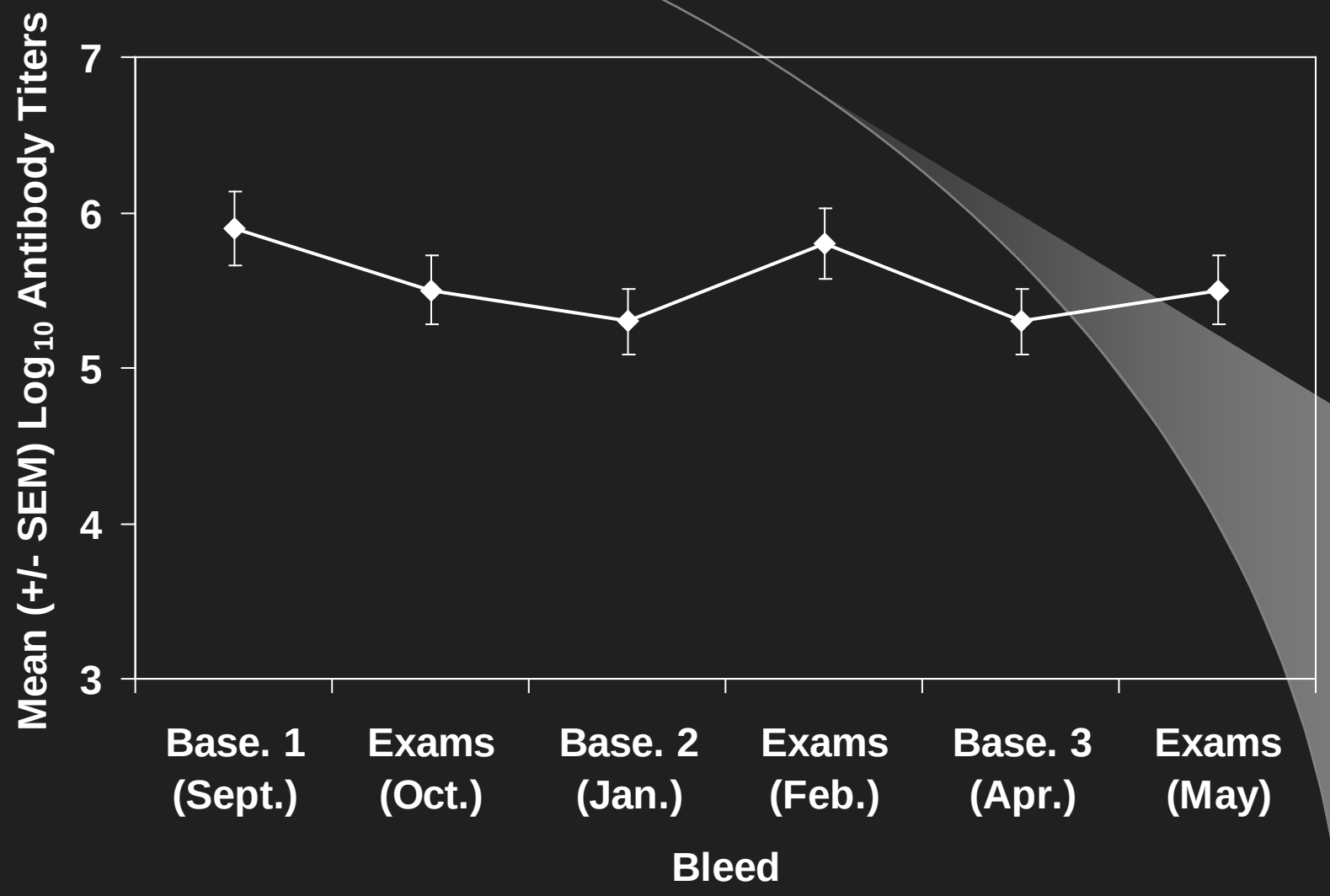
Antibody Titers to EBV



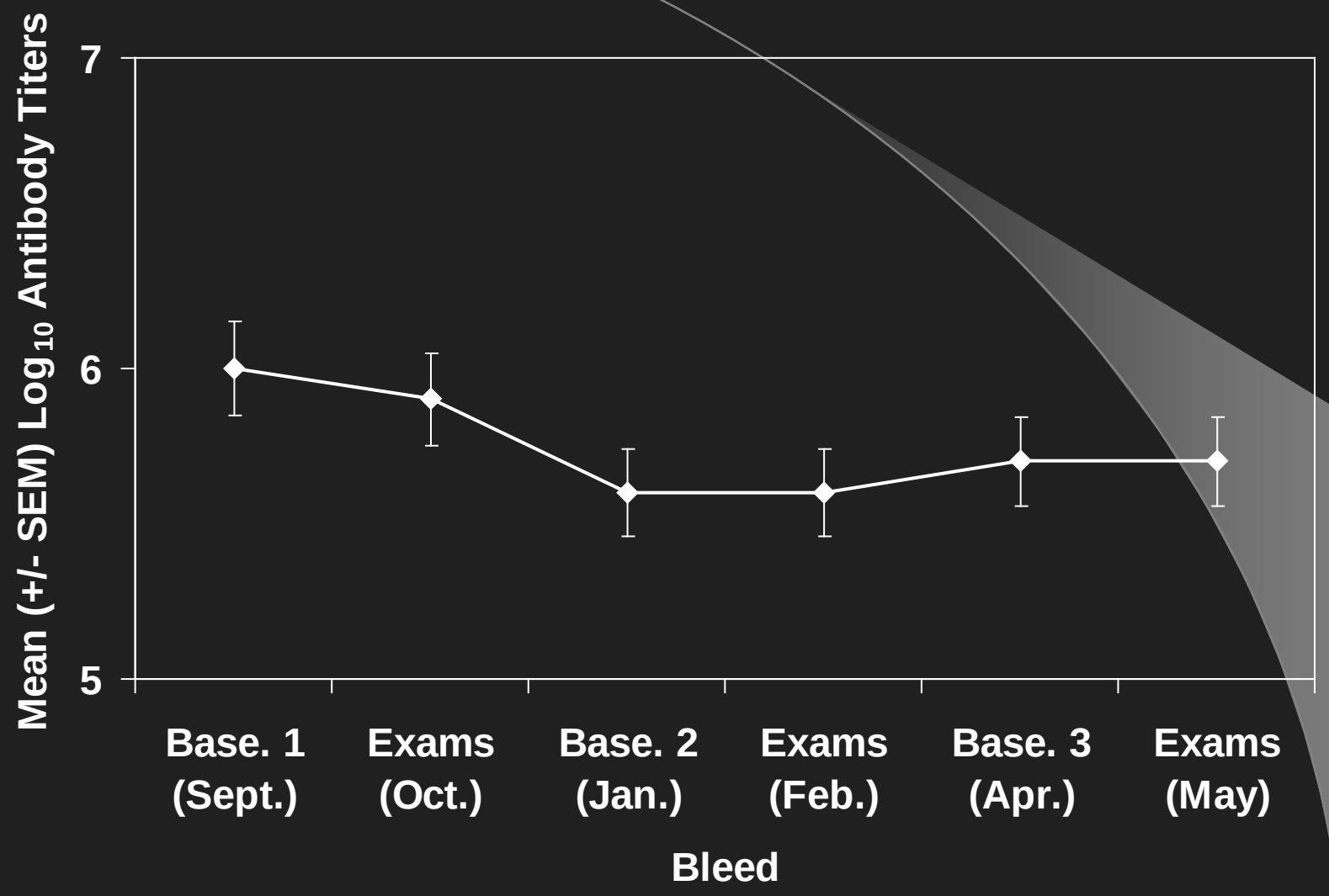
Antibody Titers to EBV Late Membrane Antigen (gp 350/300)



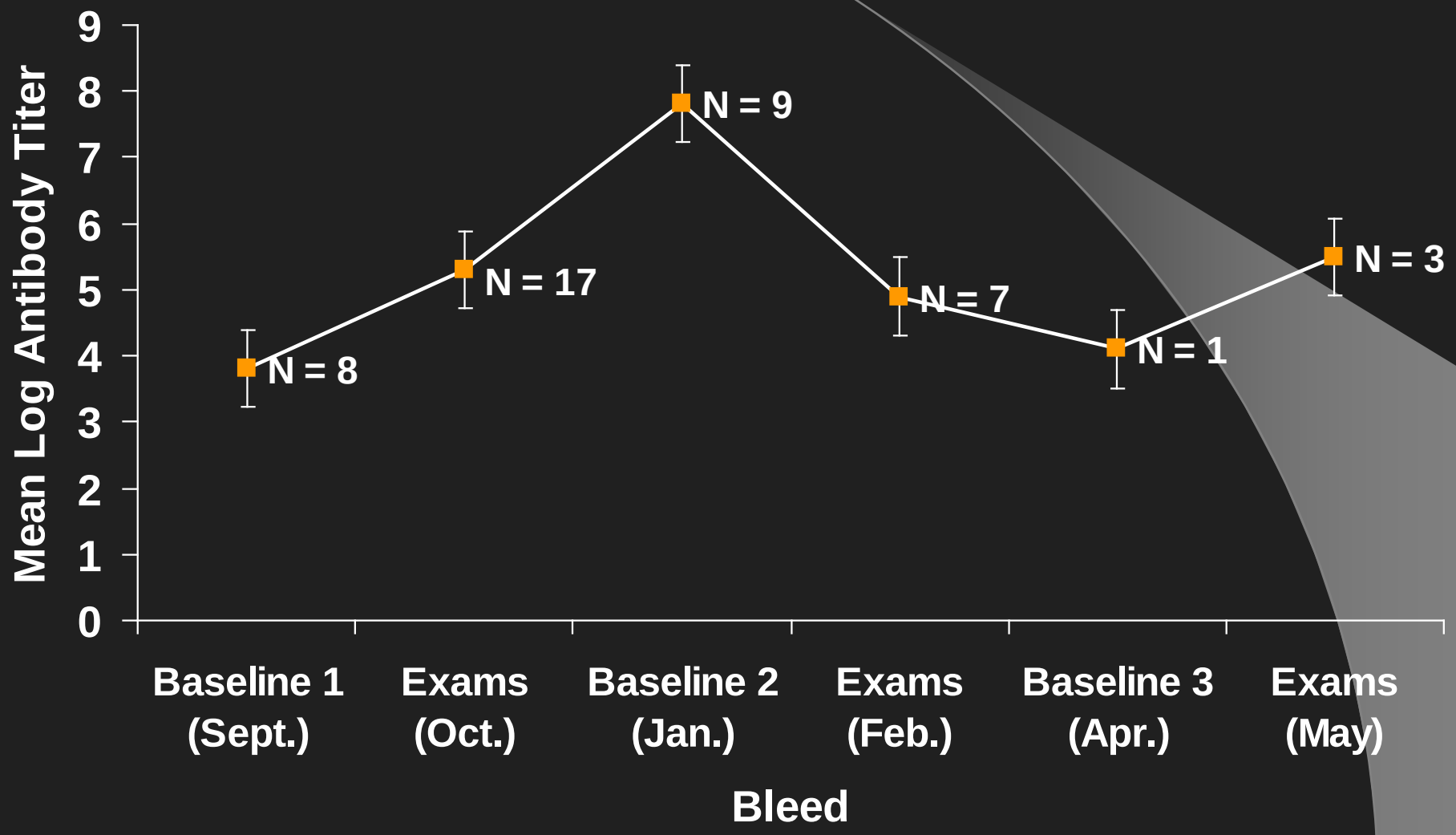
Antibody Titers to EBV EA-R (85 Kd)



Antibody Titers to EBV VCA (125 Kd)



Mean (± SEM) Log Antibody Titers to EBV EA-D (52/50 Kd), DNase



Autonomic and Glucocorticoid Associations with the Steady State Expression of Latent Epstein-Barr Virus

John T. Cacioppo

University of Chicago

Janice K. Kiecolt-Glaser, William B. Malarkey, Bryon F. Laskowski, Leigh Ann Rozlog

The Ohio State University College of Medicine

Kirsten M. Poehlmann

University of Houston

Mary H. Burleson

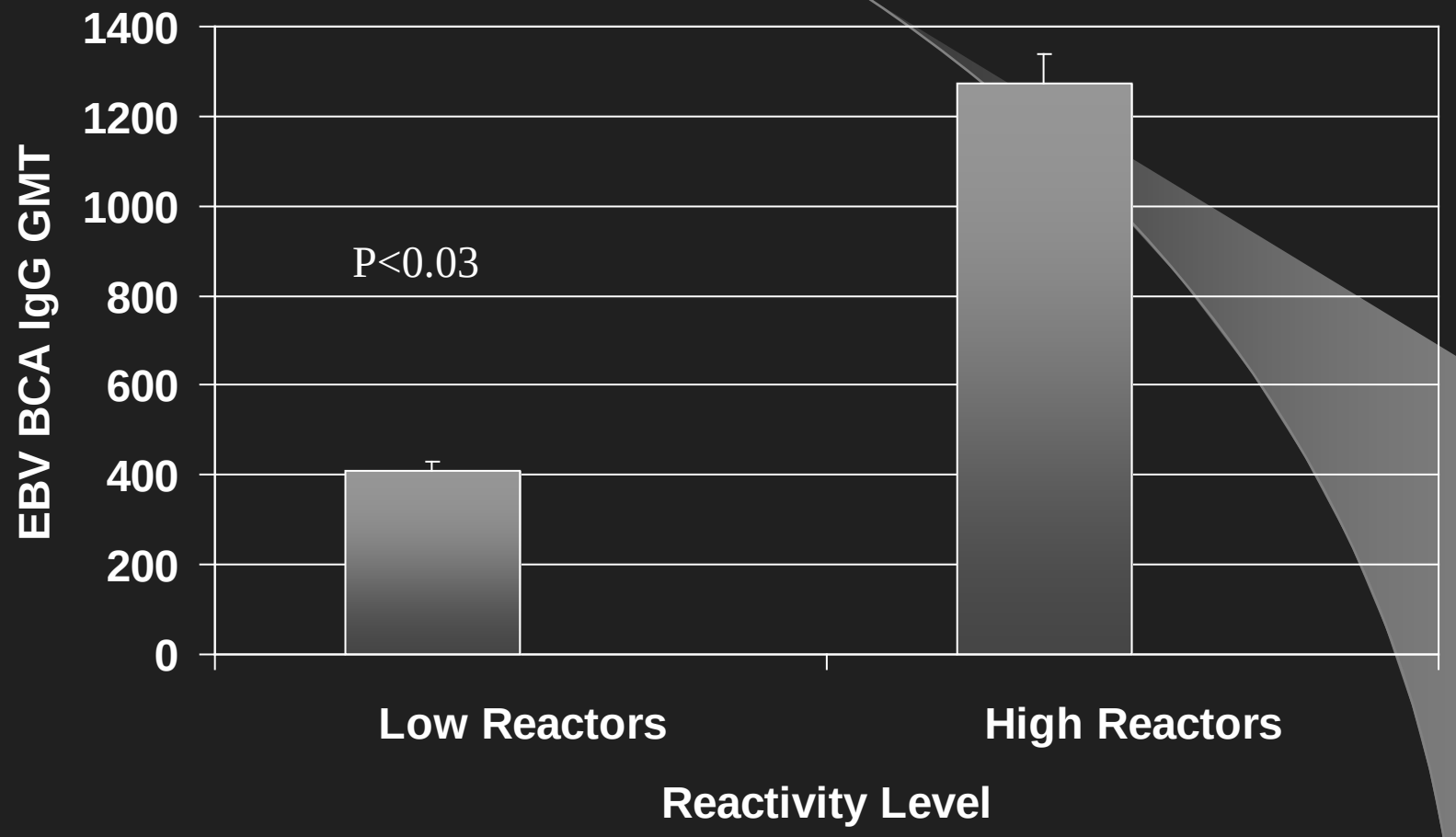
Arizona State University

Ronald Glaser

The Ohio State University College of Medicine

Hormones & Behavior **42**: 32-41, 2002

We investigated whether the steady-state expression of latent EBV in 50 elderly women differed between high and low stress reactors, as defined by sympathetic cardiac reactivity



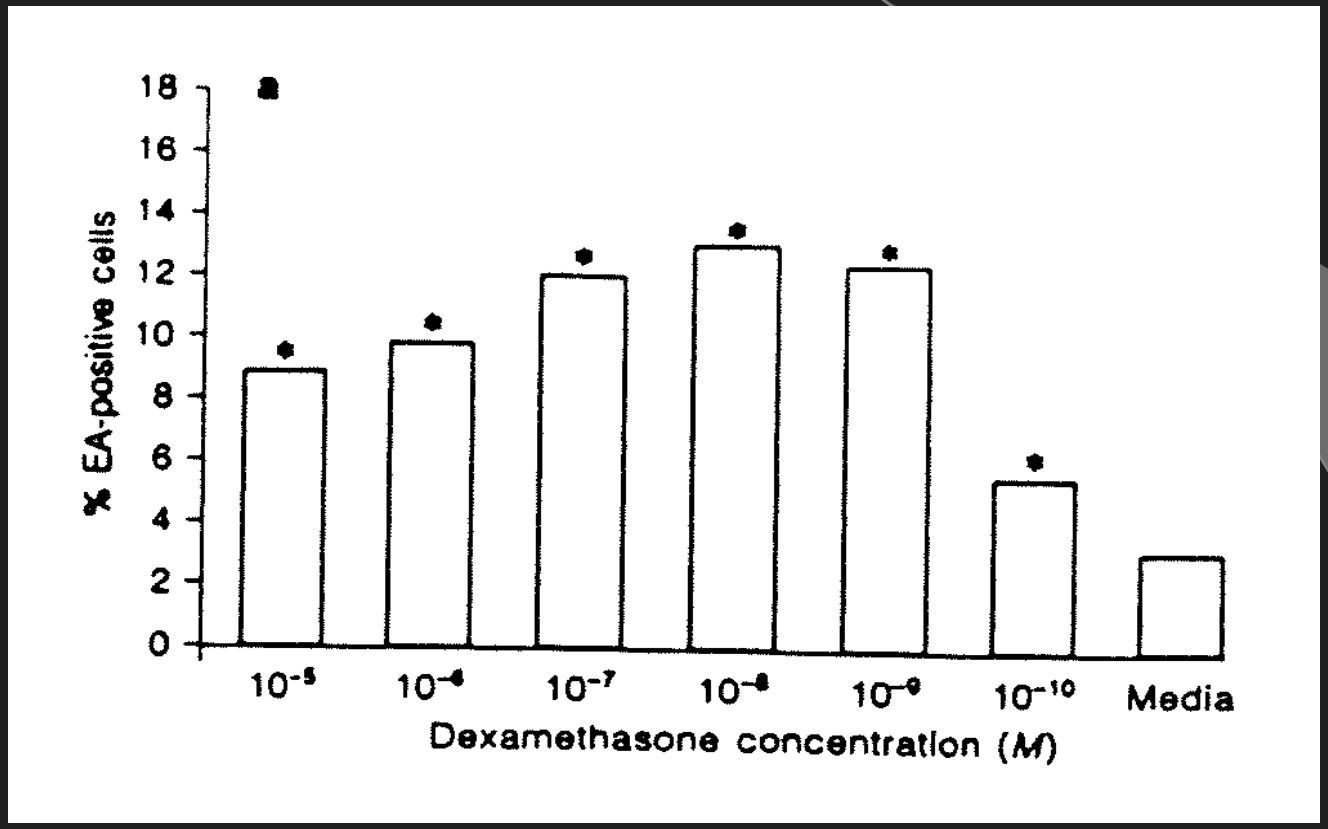
Hormonal modulation of Epstein-Barr virus replication

Glaser, R., Kutz, L.A., MacCallum, R.C., and Malarkey, W.B

Neuroendocrinology

62: 356-361, 1995

Dexamethasone and reactivation of latent EBV 48 hours after treatment

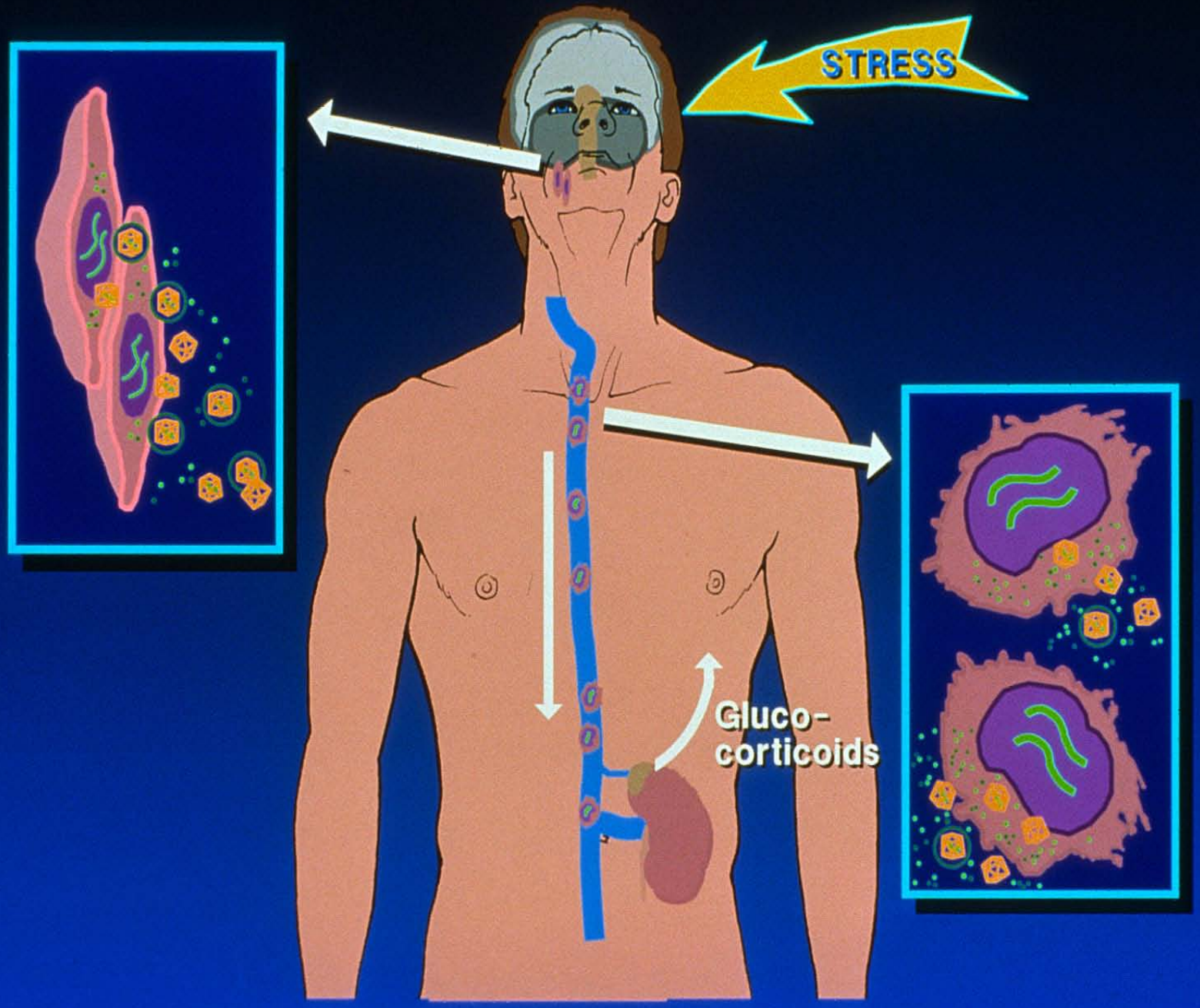


Glucocorticoids activate Epstein Barr Virus lytic replication through the upregulation of immediate early BZLF1 gene expression

Yang and Marketon, et al.,
Brain, Behavior and Immunity
24: 1089-1096, 2010

In this study, we tested the hypothesis that GCs can induce the latent EBV genome to lytically replicate through the induction of the EBV immediate early gene BZLF1 which encodes the lytic transactivator protein ZEBRA.

The results suggest that GCs, in addition to inducing stress-related immune dysregulation, can mediate latent EBV reactivation through the induction of the BZLF1


STRESS
Gluco-
corticoids

Early Proteins (Enzymes) Associated with EBV Replication

- Glaser, R., Ogino, T., Zimmerman, J., and Rapp, F. "Thymidine Kinase Activity in Burkitt Lymphoblastoid Somatic Cell Hybrids After Induction of the EB Virus." *Proceedings of the Society for Experimental Biology and Medicine* **142**: 1059-1062, 1973.
- Miller, R., Glaser, R., and Rapp, F. "Studies of an Epstein-Barr Virus-Induced DNA Polymerase." *Virology* **76**: 494-502, 1977.
- Henry, B.E., Glaser, R., Hewetson, J., and O'Callaghan, D. "Expression of altered ribonucleotide reductase activity associated with the replication of the Epstein-Barr virus." *Virology* **89**: 262-271, 1978.

Enzymes Associated with EBV Replication

- Cheng, Y.-C., Chen, J.Y., Hoffmann, P.J., and Glaser, R. "Studies on the activity of Dnase associated with the replication of the Epstein-Barr virus." *Virology* **100**: 334-338, 1980.
- Williams, M.V., Holliday, J.E., and Glaser, R. "Induction of a deoxyuridine triphosphate nucleotidohydrolase activity in Epstein-Barr virus-infected cells." *Virology* **142**: 326-333, 1985.

Frequency and Levels of Antibodies to Epstein-Barr Virus Specific DNase Are Elevated in Patients With Nasopharyngeal Carcinoma

Y.-C. Cheng, Y.-Y. Chen, R. Glaser, and W. Henle

Proceedings of the National Academy of Science
77 (10): 6161-6165, 1980.

Incidence of the Presence of Antibodies Against EBV Induced DNase in Human Serum

Patient Group	Subject	Anti EBV DNase Activity (Unit neutralized/ml serum)		
		<3	3-6	>6
Normal	101	94 (93)	7 (7)	1 (1)
Infectious Mononucleosis	58	52 (90)	2 (3)	4 (7)
Nasopharyngeal Carcinoma	49	2 (4)	1 (2)	46 (94)
Burkitt Lymphoma	47	28 (60)	10 (21)	9 (19)
Hodgkins Disease	26	18 (69)	3 (11)	5 (20)
Acute Myelocytic Leukemia	39	31 (80)	5 (13)	3 (7)
Prostate Carcinoma	98	88 (90)	2 (2)	8 (8)

Antibodies to Epstein-Barr Virus-Specific DNase and DNA Polymerase in the Chronic Fatigue Syndrome

Jones, J.F., Williams, M.W., Schooley, R.T., Robinson, C., and Glaser, R.

Archives of Internal Medicine 148: 1957-1960, 1988.

High titers of anti-EBV DNA polymerase are found in patients with severe fatiguing illness

Natelson, B.H., Ye, N., Moul, D.E., Jenkins, F.J., Oren, D.A., Tapp, W.N. and Cheng, Y-C

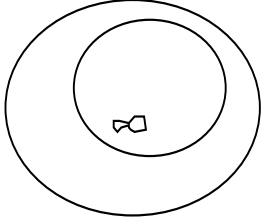
Journal of Medical Virology 42: 42-46, 1994.

EBV Antibody Patterns in Patients with Different EBV Associated Diseases

Disease	EA-R	EA-D	VCA	EBNA	DNase and/or DNA polymerase	dUTPase
EBV+ normal subjects	-	+	+	+	-	-
IM	-	+	+	+ (delayed)	-	+
BL	+	-	+	+	+	-
NPC	-	+	+	+	+	+
CFS	+	+++	+	+	+* *two studies	+ AIDS and chronic EBV infection

Possible Explanations to Explain Unique Antibody Patterns to EBV Proteins

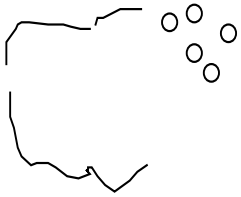
- Certain genes may be over-expressed during lytic replication in different cell types
- “Abortive reactivation” of latent virus with only some early proteins expressed in intact cells.



LATENTLY INFECTED CELL

BZLF1 ACTIVATION

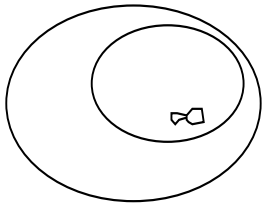
LYTIC REPLICATION



1. EXPRESSION OF β GENES
2. REPLICATION OF VIRAL DNA
3 EXPRESSION OF γ GENES
4. NEW VIRUS PRODUCTION
5. CELL LYSIS

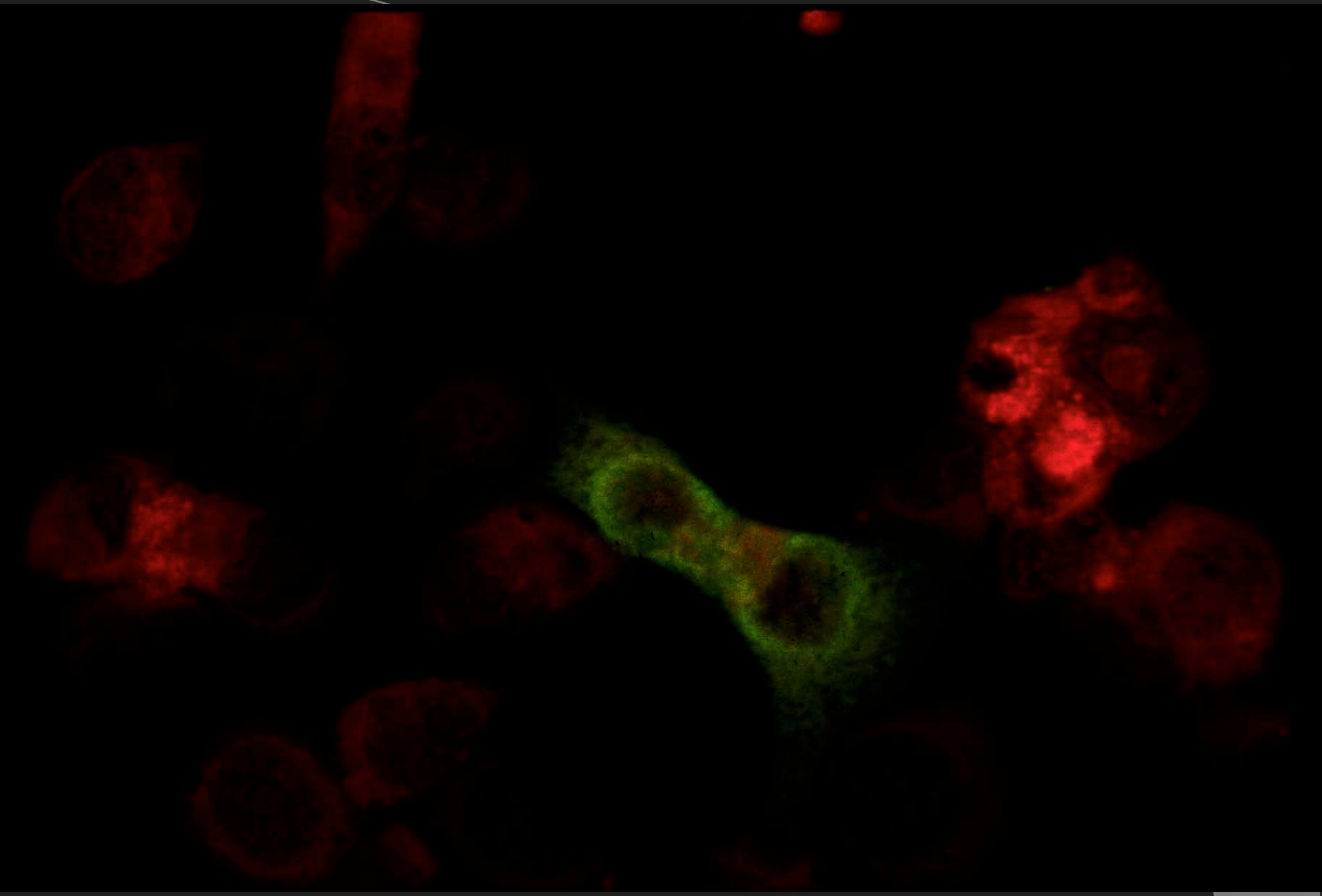
1. INCREASED ANTIBODY RESPONSE TO EA COMPLEX AND VCA
2. INCREASE IN VIRAL DNA

ABORTIVE REPLICATION



1. EXPRESSION OF β GENES or A SUBSET OF β GENES

1. INCREASED ANTIBODY RESPONSE TO DIFFERENT COMPONENTS OF EA COMPLEX
2. NO INCREASE IN ANTIBODIES TO VCA
3. NO INCREASE IN VIRAL DNA



Cloning and expression of Epstein-Barr virus-encoded dUTPase: patients with acute, reactivated or chronic virus infection develop antibodies against the enzyme

Sommer et al, J Gen Virol, 1996

Sera from EBV-negative subjects or healthy subjects latently infected with EBV did not have detectable levels of antibody to the dUTPase. Patients with mononucleosis, patients with chronic EBV infection, and patients co-infected with HIV had elevated antibody titers against the protein.

Hypothesis: GC hormones can modulate the steady state expression of latent herpes viruses, e.g. EBV. Viral proteins and/or viral particles are then synthesized. Complete virus reactivation and synthesis of infectious virus may not occur in all instances; some early viral proteins could still be encoded and synthesized. Our lab and others have shown that some of viral proteins have the ability to induce immunomodulation that result in an increase in proinflammatory cytokines which in turn can induce immunopathology/clinical symptoms that we call CFS.

Comparison of the effects of central or peripheral administration of cytokines with non-specific symptoms of sickness* (Dantzer& Kelley, 1989)

- General malaise
- Decreased activity
- Decreased social investigation
- Decreased food and water intake, weight loss
- Sleep changes
- Fever

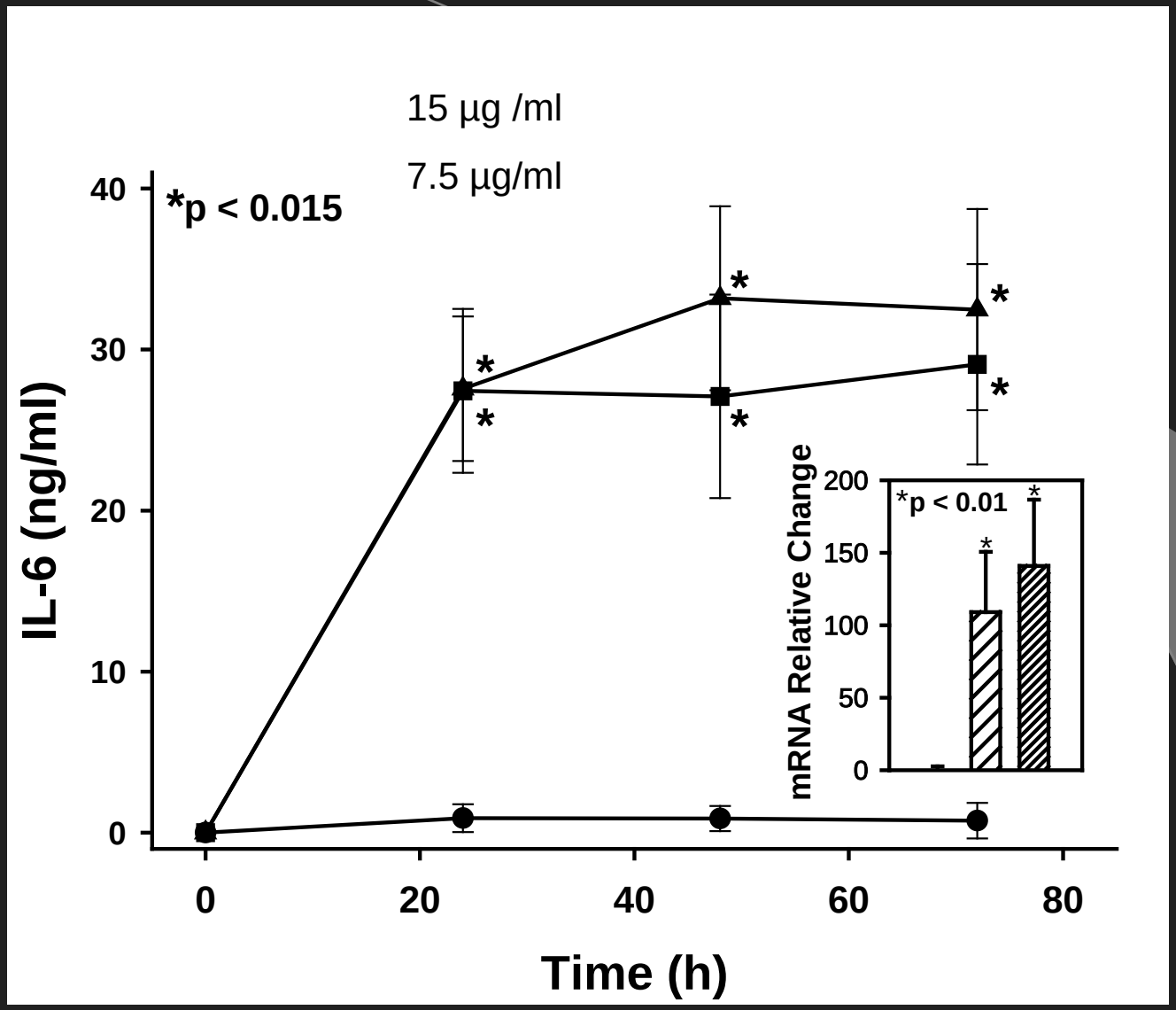
- Feeling sick
- Loss of energy or fatigue
- Loss of interest in usual activities
- Poor appetite and significant weight loss
- Sleep changes
- Fever

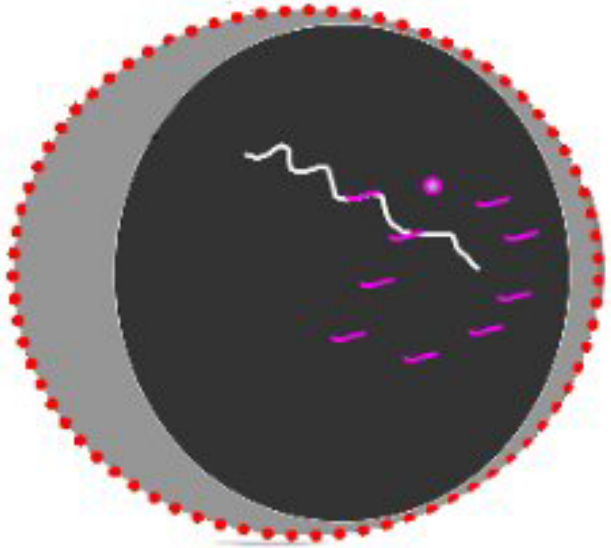
*observed in clinical studies with human subjects and/or in animal models

EBV-encoded deoxyuridine triphosphate nucleotidohydrolase (dUTPase) induces immune dysregulation: Implications for the pathophysiology of EBV-associated disease.

Virology, 346(1):205-218, 2006.

Glaser, Litsky, Padgett, Baiocchi, Yang, Chen, Yeh, Green-Church, Caligiuri and Williams

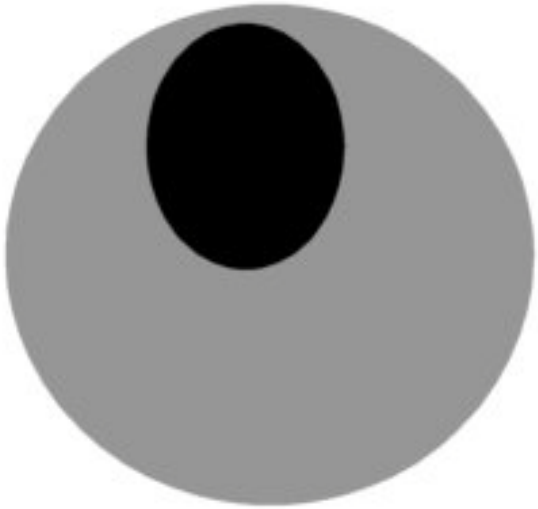

15 µg /ml
7.5 µg/ml
*p < 0.015
IL-6 (ng/ml)
Time (h)
0
10
20
30
40
0
20
40
60
80
mRNA Relative Change
*p < 0.01
0
50
100
150
200



dUTPase

TOLL LIKE
RECEPTOR 2
ACTIVATES NFkB

IL-6, IL-8,
IL-10, IL-1β,
TNF-α



CD14+
monocytes

DENDRITIC CELLS

Epstein-Barr Virus-Encoded dUTPase Modulates Immune Function and Induces Sickness Behavior in Mice

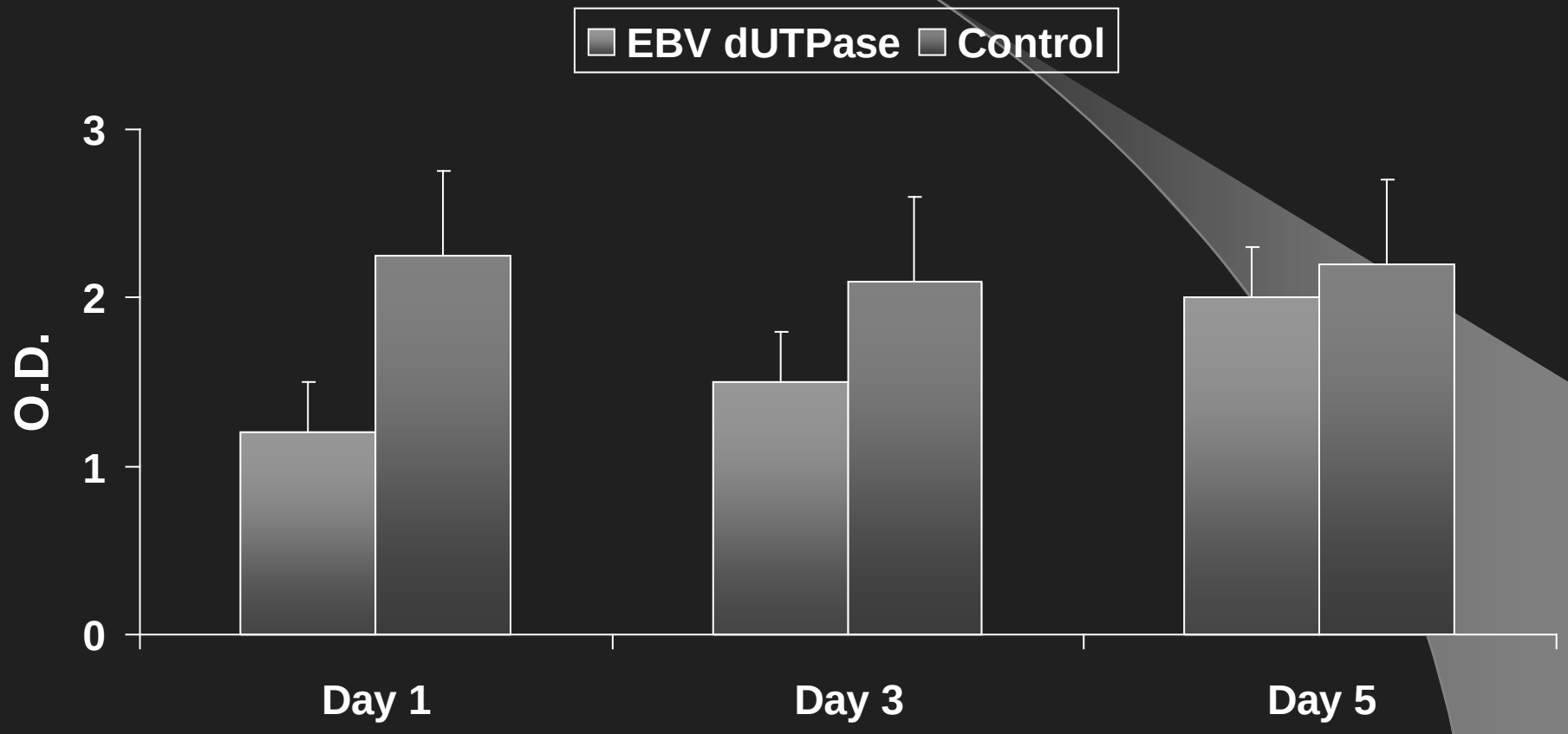
David A. Padgett, Andrew K. Hotchkiss, Leah M. Pyter, Rand J. Nelson, Eric V. Yang, Peir-En Yeh, Monica L. Litsky, Marshall V. Williams, Ronald Glaser

Journal of Medical Virology

74:442-448 (2004)

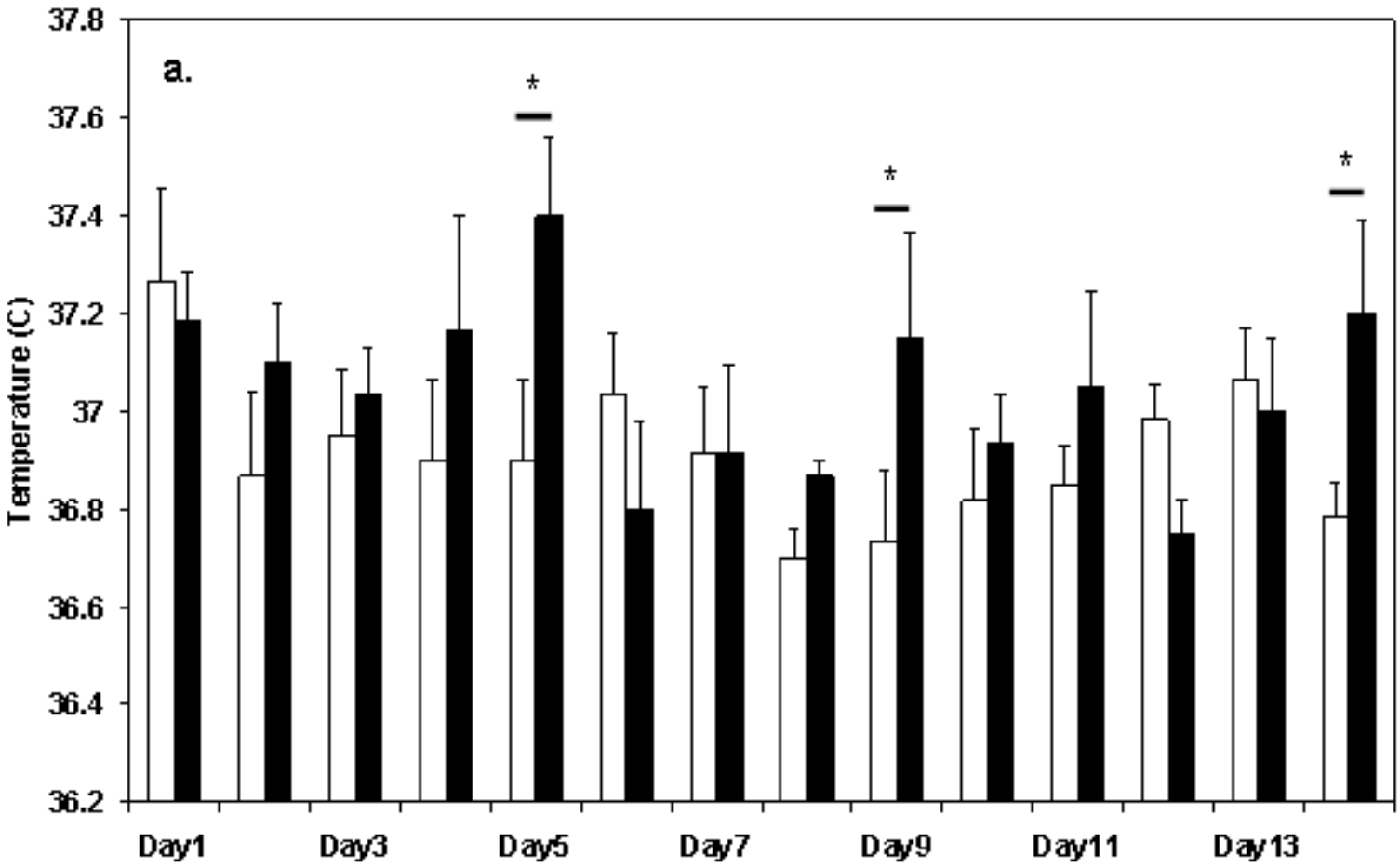
CFS Mouse Model?

Blastogenic Response (Con-A) of Lymph Node Derived Lymphocytes Obtained from Mice Treated with EBV dUTPase



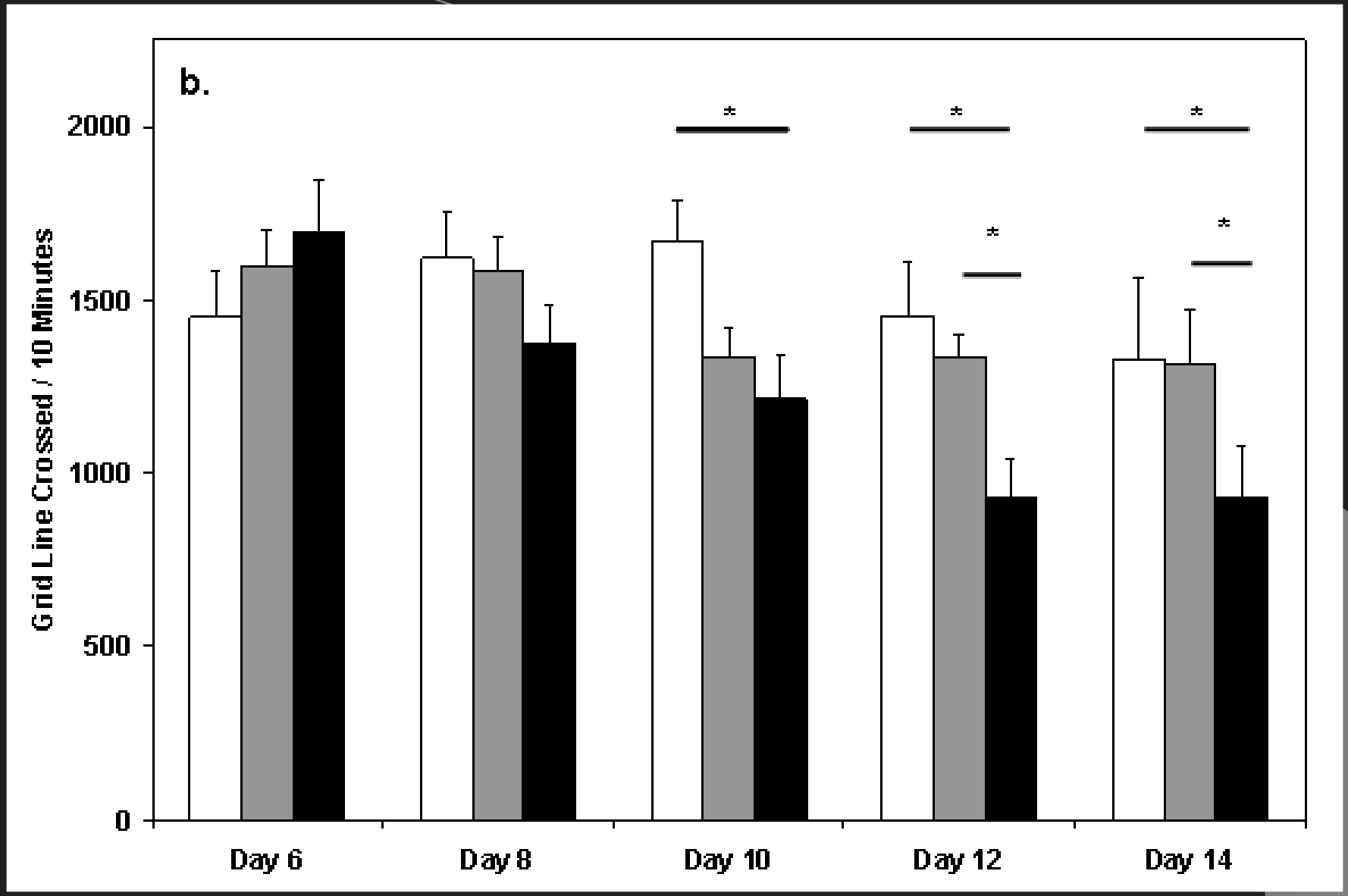
***p=<.001 Significant time effect p=<.001**

Average of 3 Experiments, N-11/group



□ Control ■ EBV dUTPase

n = 5/group



n = 5/group

Control Vehicle EBV dUTPase

Hypothesis: Abortive reactivation of latent EBV may result in the synthesis of some EBV encoded early proteins, e.g. the dUTPase, DNase and DNA polymerase in the absence of EBV DNA synthesis. This can happen because the synthesis of EBV encoded early proteins does not depend on EBV DNA synthesis. The result would be an increase in one or more early proteins but with nondetectable changes in viral DNA load. Such a reactivation pattern would be masked because of the way laboratories normally measure EBV antibody titers e.g. EA/VCA. It is possible that the usual antibody patterns to EA/VCA may not reflect what is actually happening in CFS patients. The Cacioppo paper, West Point and the PNAS Mouse Papers underline the importance that emotions have in relation to EBV latency. This hypothesis does not rule out the possibility of interaction between latent EBV, another herpesvirus(s), and/or a latent retrovirus. It is clear that there is an important role for studying stress induced immune dysregulation as a cofactor for CFS.