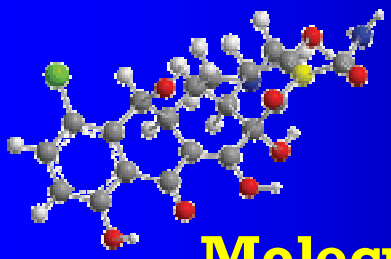


Molecular Mechanisms Driving the Current Epidemic of Chronic Disease

Trevor G. Marshall, PhD
Autoimmunity Research Foundation
www.AutoimmunityResearch.org

revised: Nov13,2006

Copy of slides available from:
www.AutoimmunityResearch.org/tm_aust_2006.pdf

Published Sources - citations

This presentation will draw upon the work of others, usually referenced by a PubMed ID number 'PMID:', and upon my own recent peer-reviewed publications. These include:

Marshall TG: VDR Nuclear Receptor Competence is the Key to Recovery from Chronic Inflammatory and Autoimmune Disease. 'Days of Molecular Medicine', 2006. http://autoimmunityresearch.org/karolinska-handout.pdf

Marshall TG: Molecular genomics offers new insight into the exact mechanism of action of common drugs - ARBs, Statins, and Corticosteroids. FDA CDER Visiting Professor presentation, FDA Biosciences Library, Accession QH447.M27 2006

Marshall TG, Lee RE, Marshall FE: Common angiotensin receptor blockers may directly modulate the immune system via VDR, PPAR and CCR2b. Theor Biol Med Model. 2006 Jan 10;3(1):1. PMID:16403216

Waterhouse JC, Marshall TG, Fenter B, Mangin M, Blaney G: High levels of active 1,25-dihydroxyvitamin D despite low levels of the 25-hydroxyvitamin D precursor - Implications of dysregulated vitamin D for diagnosis and treatment of Chronic Disease. In Vitamin D: New Research. Volume 1. Edited by: Stoltz VD. New York: Nova Science Publishers; 2006. ISBN: 1-60021-000-7

Published Sources – citations - continued

Marshall TG, Fenter BJ, Marshall FE: Antibacterial Therapy Induces Remission in Sarcoidosis (in English). JOIMR 2005;3(1):2 Available from URL http://www.joimr.org/phorum/read.php?f=2&i=107&t=107

Marshall TG, Fenter B, Marshall FE: Antibacterial Therapy Induces Remission in Sarcoidosis. Herald MKDTS 2004g; Volume III: Release 1. (The Journal of the Interregional Clinical-Diagnostic Center, Kazan, Invited paper, Special issue on Sarcoidosis. Published in Russian translation). ISSN: 1726-6149

Marshall TG, Marshall FE: Sarcoidosis succumbs to antibiotics - implications for autoimmune disease. Autoimmunity Reviews,2004; 3(4):295-3001 http://dx.doi.org/10.1016/j.autrev.2003.10.001 PMID: 15246025

Marshall TG, Marshall FE: Antibiotics in Sarcoidosis - Reflections on the First Year. JOIMR 2003; 1(3):2

Marshall TG, Mekhiel N, Jackman WS, Perlman K, Albisser AM: New microprocessor-based insulin controller. IEEE Trans Biomed Eng. 1983 Nov;30(11):689-95. PMID: 6662523

Keogh EJ, MacKellar A, Mallal SA, Dunn AG, McColm SC, Somerville CP, Glatthaar C, Marshall T, Attikiouzel J: Treatment of cryptorchidism with pulsatile luteinizing hormone-releasing hormone (LH-RH). J Pediatr Surg. 1983 Jun;18(3):282-3. PMID: 6135766

Full list of publications at http://TrevorMarshall.com/papers.htm

Disclosures, FDA applications in process

Many of the disease states described in this presentation, including neurological states, are not generally accepted as being caused by pathogens. Much of this presentation is based upon leading edge science, not on "weight of evidence." A Phase-2 clinical trial is ongoing.

Even when shown the science, and the microscopy, some experts still disagree with the existence of persistent pathogens. The FDA has now designated long-term use of Minocycline and Clindamycin in the treatment of Sarcoidosis, but not in the other indications I discussed.

Although FDA applications are current for PTLDS and Sarcoidosis, marketing approval has not yet occurred, and use of these principles is therefore 'off-label'

The following FDA OOPD applications are currently active: 05-2131, 05-2133, 05-2134, 06-2287,06-2288,06-2289, and their text should be consulted for further information

"There are very few things which we know, which are not capable of being reduced to a Mathematical Reasoning,… and where a Mathematical Reasoning can be had, it's as great folly to make use of any other, as to grope for a thing in the dark when you have a Candle standing by you."
"Of the Laws of Chance." John Arbuthnot (1692)

The primary difference between Mathematical Science and Evidence-Based Medicine is that one is definitive, and one is interpretive.

True science has no concept of "weight of evidence." An hypothesis is advanced, it is tested, and it stands until it is rejected or improved. If the problem is deterministic, it is solvable.

The Lancet, DS Grimes, & Correspondence

As an example of how Molecular and Evidence-based technologies are symbiotic, and need to work in closer cooperation, three weeks ago *The Lancet* published my answer to a question posed in July by DS Grimes: "Are statins analogues of Vitamin D?" (*Lancet* 2006; 368:83-86)

Dr Grimes had derived his hypothesis from the following observations:

1. Vitamin D is good for you
2. Statins are good for you
3. Therefore, statins must be similar to Vitamin D (my paraphrase)

This 'Evidence-based' dilemma is easily solved by Molecular Biology, as I will show later in my presentation… (Lancet 2006; 368:1234)

Biochemists are also failing…

A search of PubMed shows an average of one paper a day is currently being published about the 'VDR', the ultimate target of the seco-steroid hormones we call the 'Vitamins D', yet the insights resulting from that knowledge are not being communicated through to the physicians who are in the front lines…

One objective of this presentation is to share what we now know about the Vitamins D, and how they affect both the immune system, and endocrine homeostasis…

Th1 Inflammatory Disease

Inflammation which results from a significant increase in the expression and activity of the cytokine Interferon-gamma is called "Th1 inflammation"

Interferon-gamma is a paracrine cytokine, not a endocrine hormone, and it does not circulate in the bloodstream. It can be measured in the inflamed tissue, but is of limited use as a clinical diagnostic marker.

Consequently, a number of other paracrine cytokines have historically been measured in order to try and infer whether a patient is presenting with type Th1 inflammation. None of the other cytokines are specific, however, and this has led to considerable confusion.

In 2001 we noted that Interferon-gamma catalyzed (30x) the formation of a seco-steroid which did circulate as a hormone, 1,25-dihydroxyvitamin-D, and we have used that marker in our ongoing research.

Th1 Inflammatory Diagnoses

Most diagnoses commonly thought to come from an 'autoimmune' pathogenesis are of type Th1. These range from Anorexia Nervosa, through Diabetes and Rheumatoid Arthritis, to Sarcoidosis, MS and ALS.

Physicians treating Th1 conditions with our antibacterial protocol have also observed that the following neurologic manifestations resolve as the patient recovers:

- Aggression (sometimes called "Lyme Rage")
- Mild Paranoia
- Mild Obsession and Compulsion (including OCD)
- Loss of memory
- Loss of cognitive ability / attention disorders
- Bipolar disorders and Suicidal ideation

Our Phase 2 study

During the past 5 years we have conducted an observational, adaptive, open-label Phase 2 study of an antibacterial therapy in a variety of Th1 diagnoses.

"by enabling more trials to be adapted based on knowledge about gene and protein markers .. trial designs .. can tell us more about safety and benefits of drugs, in potentially shorter time frames, exposing fewer people to experimental treatments, and resulting in clinical trials that may not only be more efficient but are more attractive to patients, and their physicians"

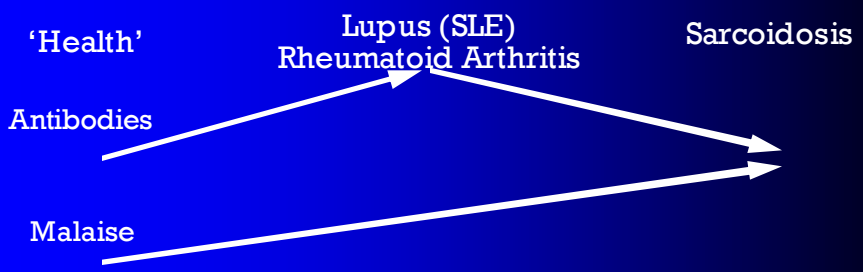
FDA Deputy Commissioner for Medical and Scientific Affairs, Dr Scott Gottlieb.

"2006 Conference on Adaptive Trial Design"
http://www.FDA.gov/oc/speeches/2006/trialdesign0710.html

Why 'antibodies to self' are not causative of the advancing disease processes

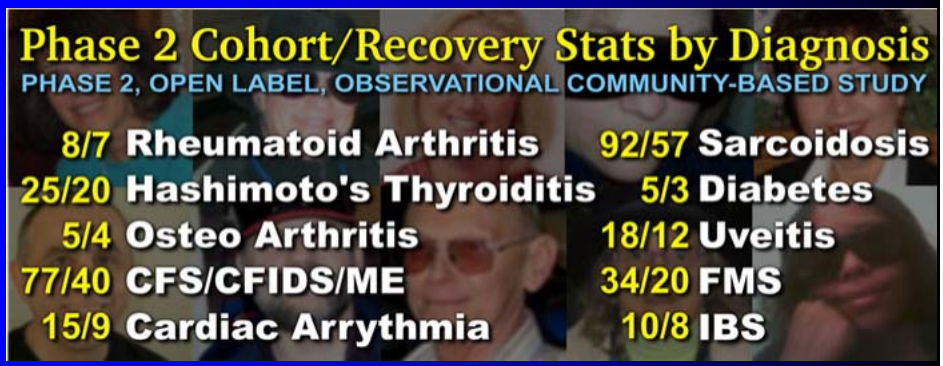
Pasteur said
"In science, chance favors the prepared mind"

We noted that a number of sarcoidosis patients had case histories involving phases where antibodies were clinically recorded, but where the antibodies disappeared as the disease progressed to sarcoidosis…



In 2004 we published our initial results, in the Th1 syndrome 'Sarcoidosis', in JOIMR and 'Autoimmunity Reviews'

My Karolinska presentation (for DMM 2006) gave the following figures for the recovery rate of key 'autoimmune' diagnoses, extracted from the Phase 2/3 reports (as of May 2006)



So how complex a Therapy is needed to address all these different Th1 Diagnoses?

Step 1: Remove all sources of exogenous Vitamin D

Step 2: Activate the VDR with Olmesartan

Step 3: For 3 months administer Demeclocycline or Minocycline q48h, increasing dose 25 to 100mg

Step 4: For 9 months add second antibiotic, either pulsed, low-dose, Azithromycin or Clindamycin

Step 5: thereafter, until complete recovery, administer pulsed, low-dose, 3 antibiotic combo Minocycline+Azithromycin+Clindamycin

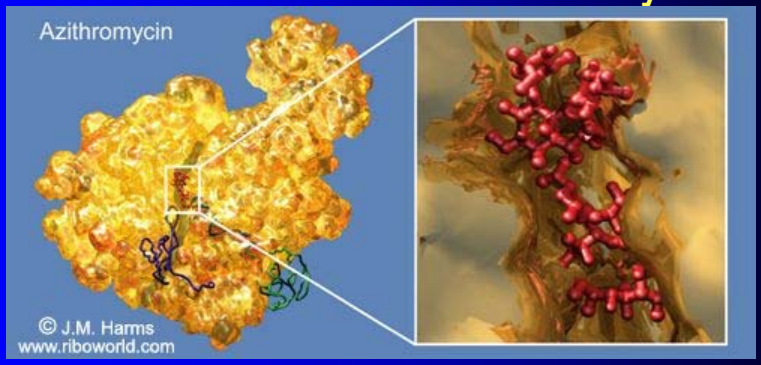
WARNING: Any one of the above steps may cause cell apoptosis with an intensity requiring emergency-room care. Immunopathology must be respected.

Steps needed to reduce systemic damage as intra-phagocytic pathogens are killed by the innate immune system:

Step 1: Apply a VDR agonist, Olmesartan, which also blocks Angiotensin II, Type 1 receptors, reducing collagen deposition (PMID: 16635409)

Step 2: Limit the Antibiotic Dosage so that the patient can manage the immunopathology

Low Dose abx blocks Protein Synthesis



1) The rate of bacterial death is controlled by inhibiting protein synthesis, using only intermittent, low-doses, of **bacteriostatic** antibiotics

2) One bacterium weakened if just one abx molecule is bound in one ribosome – low doses proportionally control the rate of bacterial death

So why??

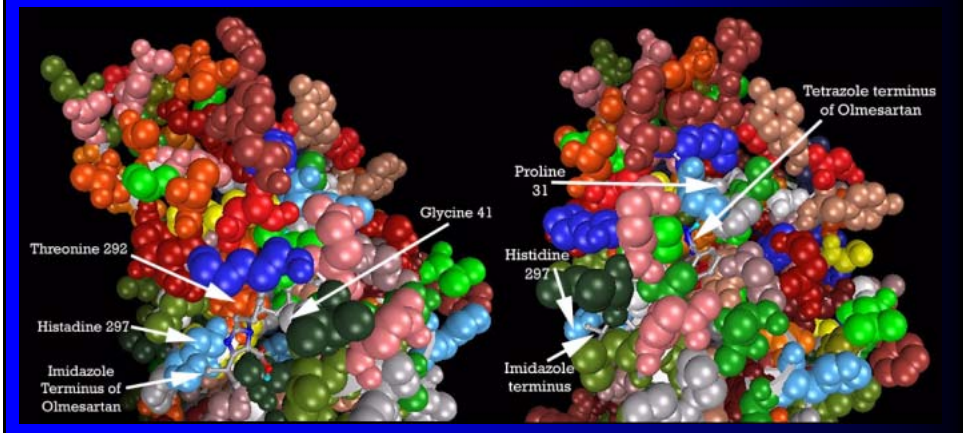
Why does this intervention work?

Why did previous antibiotic therapies not induce recovery?

This intervention works because…

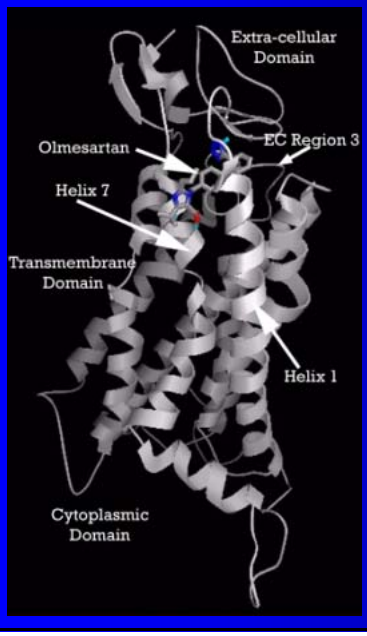
1. It is based on knowledge derived from a rigorous theoretical model based on Molecular Genomics
2. It recognizes that 'autoimmune'disease is caused by a defect in **innate** immunity, and not by 'antibodies to self'
2. It recognizes that the VDR, long thought to be 'just' associated with 'vitamin D', is actually at the heart of innate immunity
3. Sequencing of the Genomes of Bacteria and Viruses has led to an understanding of how the species interact in a chronic environment, and how an intra-phagocytic infection progresses. Many bacterial genomes have plasmids, unaffected by conventional antibiotics, which are persistent and prolific
4. Recognizes neo-natal pathogens persist in brain

G-Protein Coupled membrane Receptor- GPCR



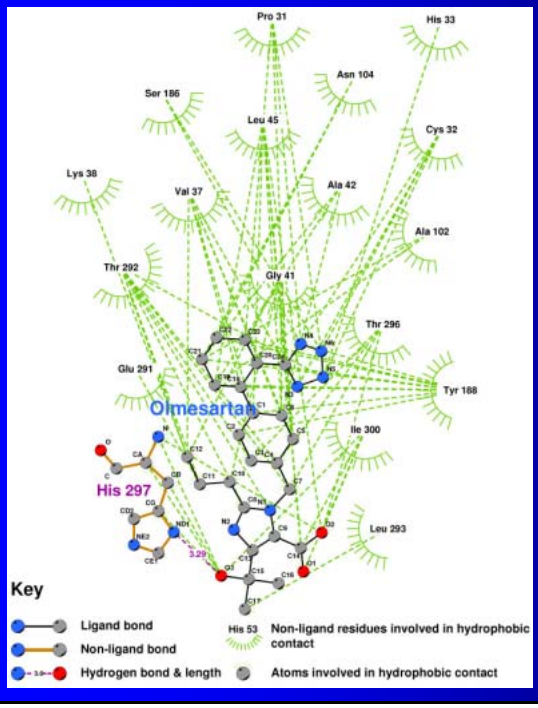
Marshall TG, Lee RE, Marshall FE: Common angiotensin receptor blockers may directly modulate the immune system via VDR, PPAR and CCR2b. Theoretical Biology and Medical Modelling. 2006 Jan 10;3(1):1 PubMed ID:16403216

G-Protein Coupled membrane Receptor- GPCR



In order to make it easier to see the structure of very large proteins, a representation which highlights helices, folds, and flaps, has been developed. We let the computer remember where each atom is located, and focus on the overview. The previous slide showed just the upper right hand corner of this same GPCR, but here the ARB can be more clearly seen. This protein is the CCR2b receptor, which allows monocytes to migrate to regions of infectious and physical trauma.

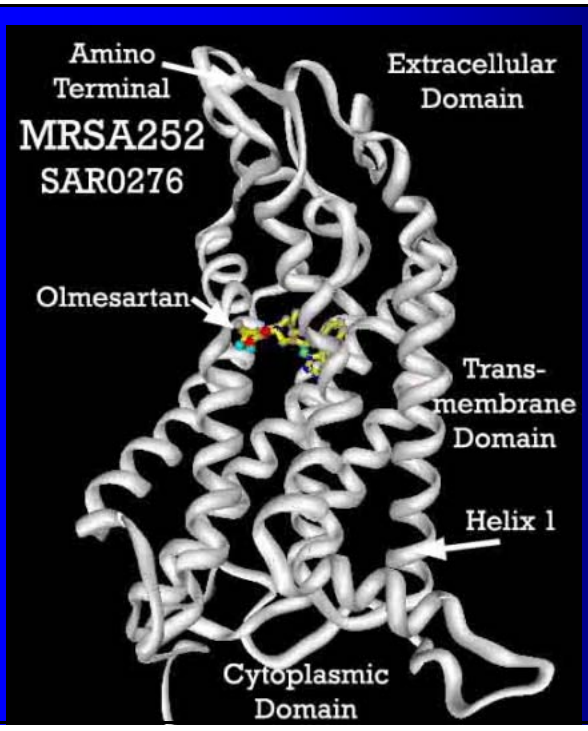
Some HIV strains can enter the phagocyte through CCR2b.



2-dimensional Molecular Representations

Here is the same ARB, in the same CCR2b binding pocket, showing the detailed atomic interactions

Agonism or Antagonism?



SAR0276 GPCR from MRSA252

The biggest surprise was the high affinity of the ARBs and Statins for VDR and PPAR-gamma, Nuclear Receptors which are key to the immune system.

While it was reasonable that these very flexible, highly polar, ligands (ARBs and Statins) might very well have an affinity for GPCR membrane receptors other than AG2R1, their affinity for the Nuclear receptors was a surprise.

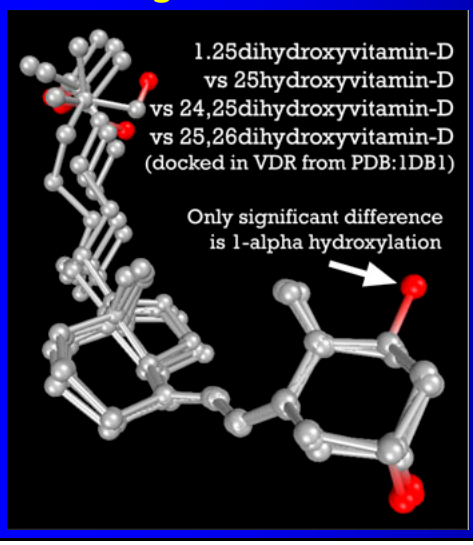
Nuclear Receptor Type 1 Family

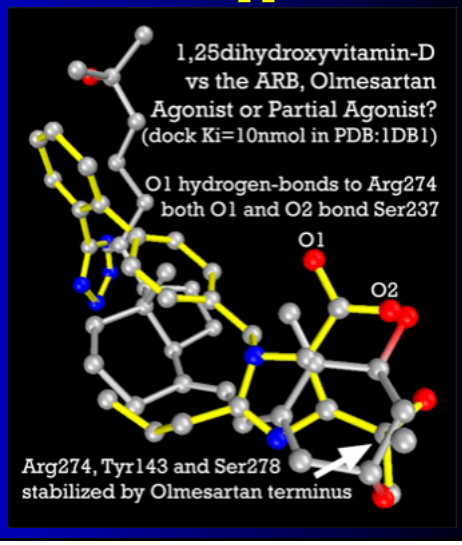
Key Nuclear Receptors with known structure models:

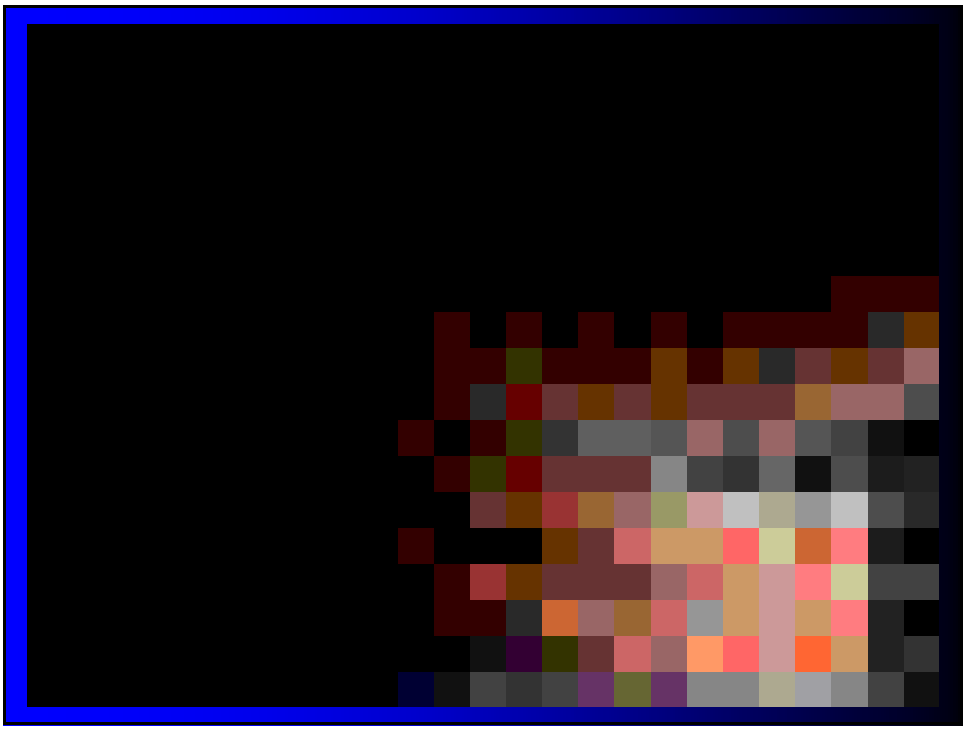
VDR (Vitamin D Receptor)	Progesterone Receptor
PPAR-alpha Receptor	Androgen Receptor
PPAR-gamma Receptor	Estrogen Receptor
GCR (glucocorticoid receptor)	Thyroid-alpha-1 Receptor
MCR (mineralcorticoid receptor)	Thyroid-beta-1 Receptor

The VDR (Vitamin D Receptor)

VDR is key to innate immunity, responsible for TLR2, TLR4, Cathelicidins, and beta-Defensins.
Exogenous Vitamin D is **immunosuppressive**







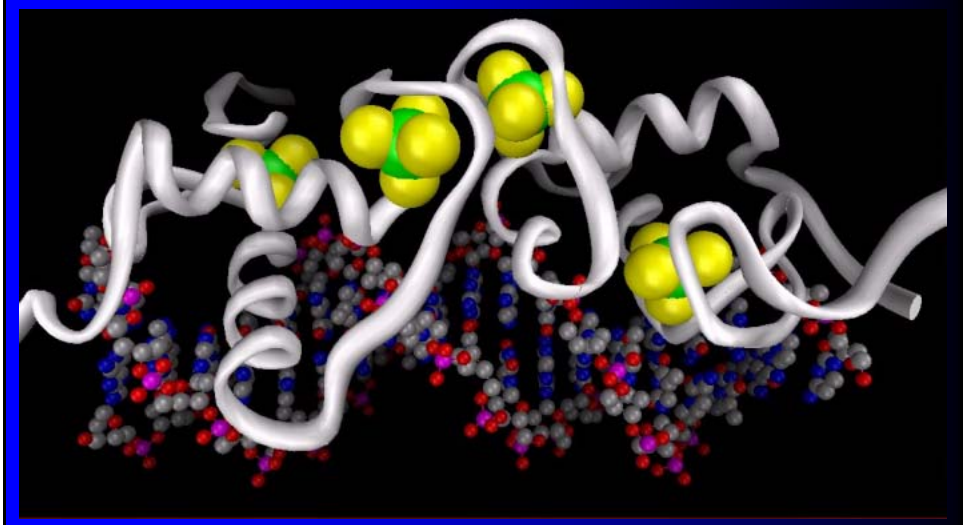
Of .. Nuclear Receptors, Homodimers, Heterodimers, Co-activators, Interdependence and Redundancy…

Nuclear Receptors are responsible for transcription of DNA genes to strands of mRNA, which are then translated (in the ribosomes) into proteins.

A simplified set of 'Flash' animations, which visually explain the transcription process, can be found online at URL: http://www.johnkyrk.com/

Redundancy and Complexity

Close-up view of GCR Homodimer 'zinc fingers'



PDB: 1R40, PubMed ID: 7664096

Estimated Ki for ARBs and Statins into NRs

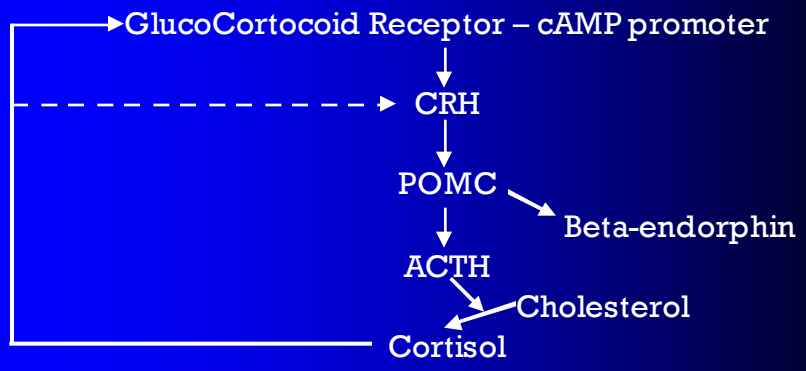
Drug est. Ki	VDR	PPARg	PPARa	GCR	MCR	PR	AT	BT
Candesartan	30	61	3	6	16	7	0.4	0.7
Irbesartan	10	6	0.9	0.8	47	4	6	0.5
Losartan	74	3	4	4	2	0.6	2	0.5
Olmesartan	10	12	3	1	4	0.3	28	2
Telmisartan	0.04	0.3	0.7	2	no	no	no	no
Valsartan	14	12	26	10	2	4	6	1
Atorvastatin	no	4	2	1	no	no	no	no
Fluvastatin	no	12	1	3	8	13	36	2
Lovastatin	10	0.2	19	15	2	0.5	2	0.3
Pravastatin	62	21	2	8	6	2	80	0.3
Rosuvastatin	no	24	18	7	3	14	no	0.6
Simvastatin	4	0.3	4	2	2	0.4	5	0.3

Steroid Activity in the Key Nuclear Receptors

Calculated Ki (nmol)	VDR	PPAR gamma	PPAR alpha	GCR	MCR	Proges-terone	Andro-gen	Thyroid alpha 1	Thyroid beta 1
PDB model >	1DB1	1FM9	1I7G	1P93	2A3I	1A28	1T5Z	1NAV	1XZX
Cortisol (steroid)	2	3	0.4	0.5	0.07	0.2	0.4	16	1
T4 (thyroxine)	60	5	0.8	3	No	51	No	0.9	0.3
Vitamin D3	0.3	3	0.7	0.04	1	0.5	2	0.02	0.02
25-hydroxy	0.07	0.5	0.4	0.03	0.9	0.1	2	0.02	0.003
24,25-dihydroxy	0.05	0.4	0.3	0.03	4	0.3	1	0.004	0.001
25,26-dihydroxy	0.1	0.2	0.4	0.05	2	0.1	1	0.02	0.003
1,25-dihydroxy	0.03	0.5	0.5	0.04	1	0.1	2	0.006	0.002

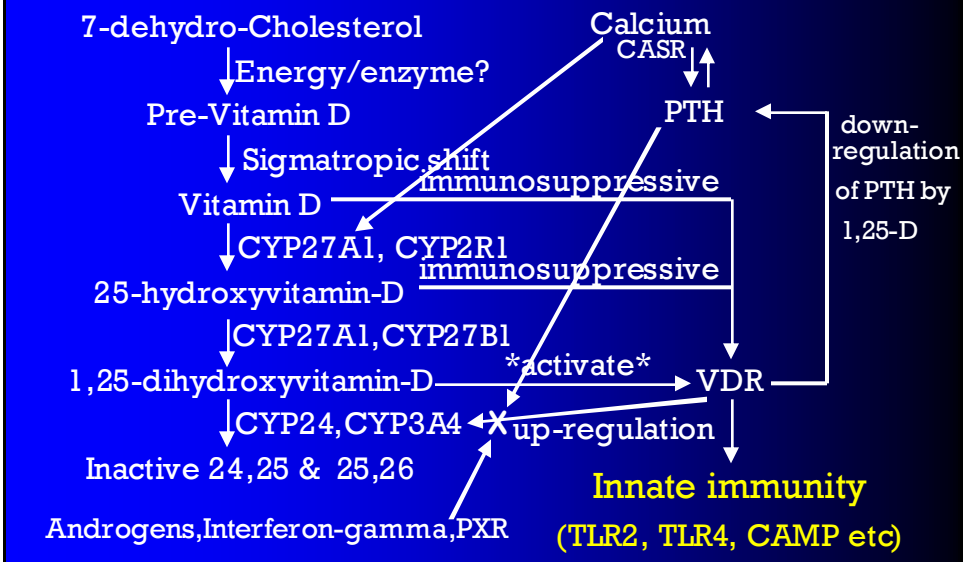
Taken from: Marshall TG: Vitamin D Metabolites affect GCR and Thyroid Nuclear Receptors. *Nuclear Receptors – Bed to Bedside*, Nov 1-5, 2006

Hypothalamic-pituitary-adrenal axis



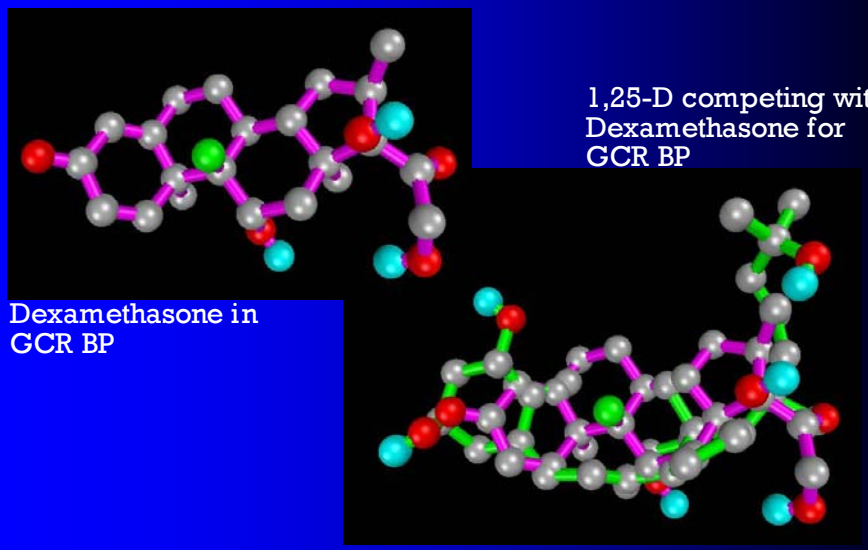
GCR = Glucocorticoid Receptor (249 aa)
CRH = Corticotropin Releasing Hormone (41 aa)
POMC = pro-opiomelanocortin (240 aa)
ACTH = Adrenocorticotropic Hormone (39 aa)
References: eg: PMID 9099914, 11754985

'Vitamin' D Steroid Metabolism



PMID:15574355, 16497887, 9099914, 11754985, 9215298, 15225756, 12914530, 15225763, 16524720, 15630458 10/23

Vitamins-D compete for GCR binding pocket



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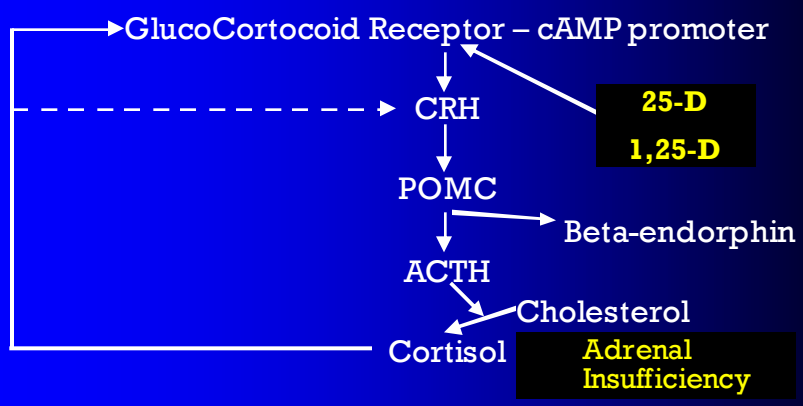
Vitamins-D perturb Cortisol homeostasis



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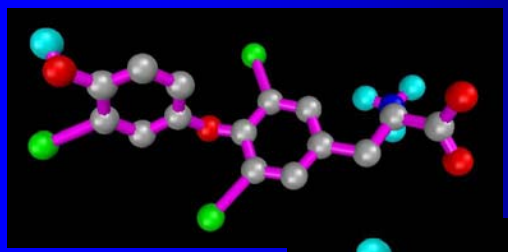
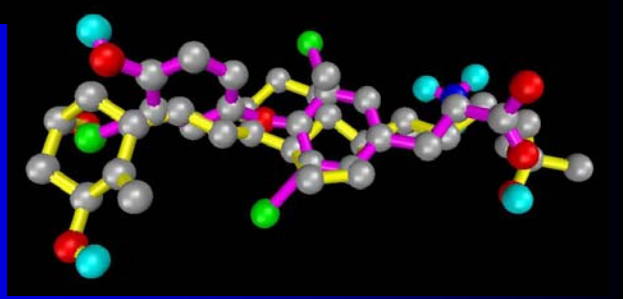
ACTH = Adrenocorticotropic Hormone (39 aa)
References: eg: PMID 9099914, 11754985

Vitamins-D compete for Thyroid binding pocket



1,25-D competing with T3 for alphaThyroid BP

T3 in alphaThyroid BP



Taken from: Marshall TG: Vitamin D Metabolites affect GCR and Thyroid Nuclear Receptors. *Nuclear Receptors – Bed to Bedside*, Nov 1-5, 2006

Vitamin D in Bone Remodelling

The calcium metabolism in *homo sapiens* is primarily controlled by the ParaThyroid Hormone (PTH) in conjunction with the Calcium Sensing Receptor (CASR), in the kidneys.
PMID:9920407,11857922,15775064

The Cannabinoid Receptors (GPCR) play a key role in bone remodelling and bone mass (PMID:16772520)

Sex hormones are also players (PMID:12017554)

Elevated levels of 1,25-dihydroxyvitamin-D, such as we have found to be associated with Th1 immune disease, actually encourage osteoclastic actions (breakdown of bone) (PMID:10782361,14988469)

Public-Health Consequences of Regarding 'Vitamin D' as a 'Vitamin'

Sometime during the 20th Century we began to view 'Cholecalciferol' as a 'Vitamin' rather than recognize its steroidal and hormonal activity.

We put the seco-steroid 'Cholecalciferol' into the food chain, in a futile attempt to eliminate the rare disease, Rickets. As physicians, you would know the side effects from administration of steroids.

The CDC now says we are heading towards half of all US seniors being Diabetic by the year 2050 ☹

Oh – don't steroids often induce obesity? ☹

Marshall TG: **Are statins analogs of vitamin D?.** Correspondence to Grimes, DS. ***The Lancet* 2006**; 368:1234

REUTERS

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U.S. children grow bigger bellies

Mon Nov 6, 2006 9:25am ET

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[-] Text [+]

WASHINGTON (Reuters) - American children and teens are growing ever-fatter tummies, a bad sign that means they are at even more risk of heart disease and diabetes, U.S. researchers reported on Monday.

They found that the belly fat of children and teenagers had increased by more than 65 percent since the 1990s -- directly in line with rising obesity rates.

But belly fat is more dangerous than general weight gain, because abdominal and visceral fat -- found surrounding the internal organs -- is more clearly and strongly linked with disease than general body fat.

Dr. Chaoyang Li of the Centers for Disease Control and Prevention, Dr. Stephen Cook of the University of Rochester School of Medicine and Dentistry in New York and colleagues examined data from several national surveys of health and fitness taken by the federal government.

They found that 10.5 percent of boys and girls had too much abdominal fat in 1999, as measured by waist circumference. This grew to 17.4 percent of boys and 17.8 percent of girls in 2004, they reported in the journal Pediatrics.

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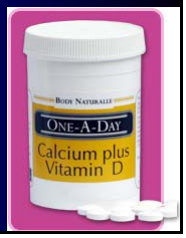
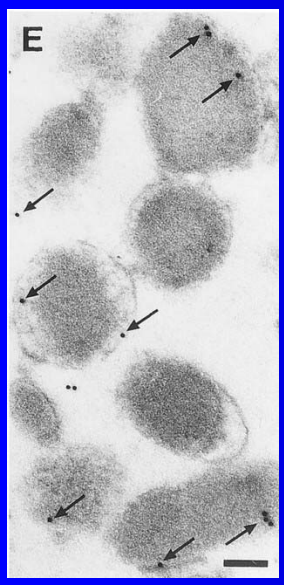


Bacterial Pathogens LIVING in the Phagocyte - a new ballgame altogether…

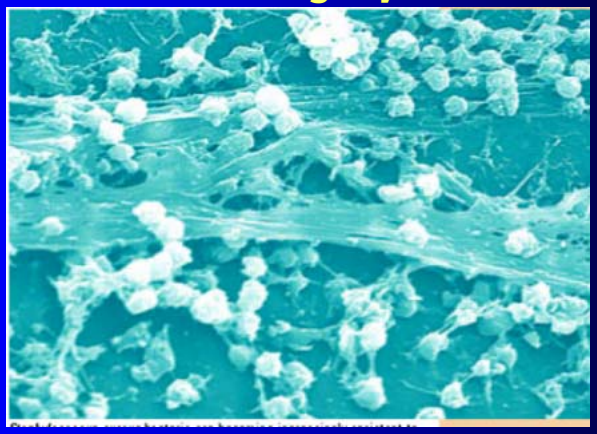


Nilsson, et al,

"Presence of Rickettsia Helvetica in granulomatous tissue from Patients with Sarcoidosis"

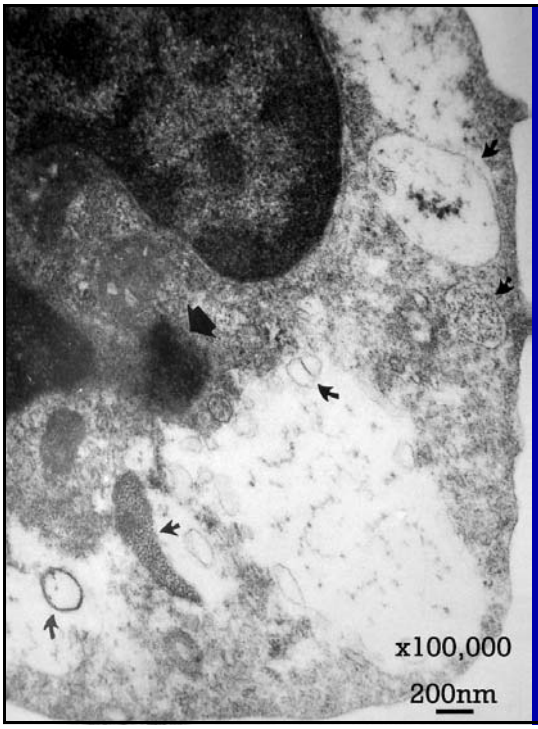
Journal of Infectious Diseases, 2002: 185: 1128

Protection from Phagocytosis - Biofilms



From: "The Microbial Resistome"..

Staph aureus are becoming increasingly resistant to B-lactam antibiotics. They secrete sticky-looking substances called biofilms .. Source: CDC



The Wirostko Studies

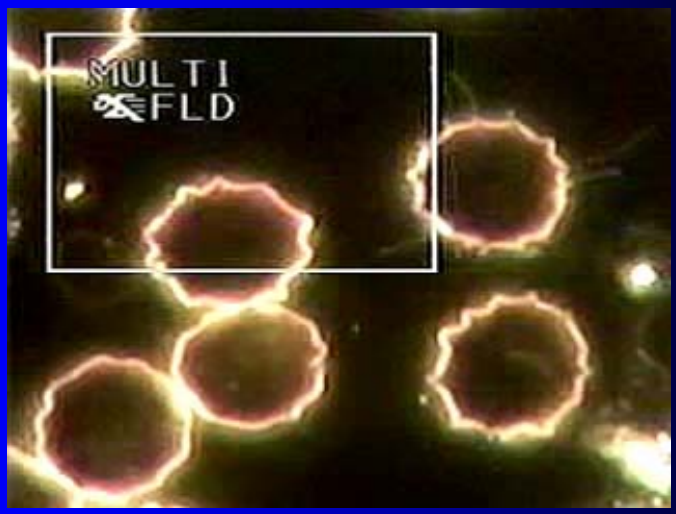
TEM photograph taken of a monocyte from the vitreous of the eye of a sarcoidosis patient showing hundreds of tiny coccoids (in colonies) have parasitized this phagocyte(also JRA, Crohn's)

The very phagocytes which are supposed to kill bacterial pathogens are providing safe harbor for them

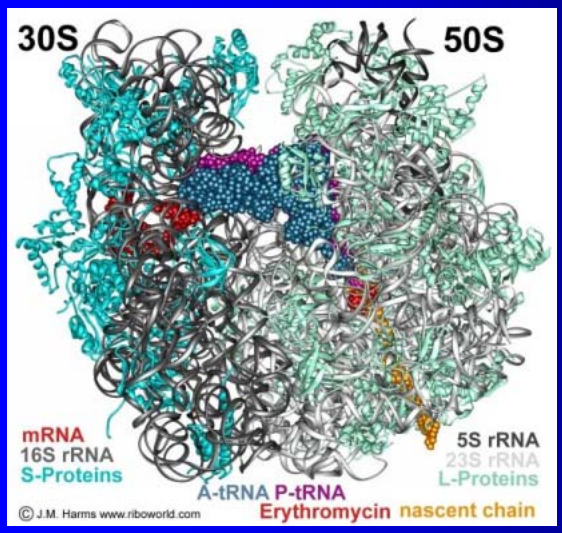
Bacteria are not being killed – therefore there are no antibodies being formed.

The more advanced the infection, the fewer the antibodies…

Phase Contrast Optical Imaging of PinPrick Blood

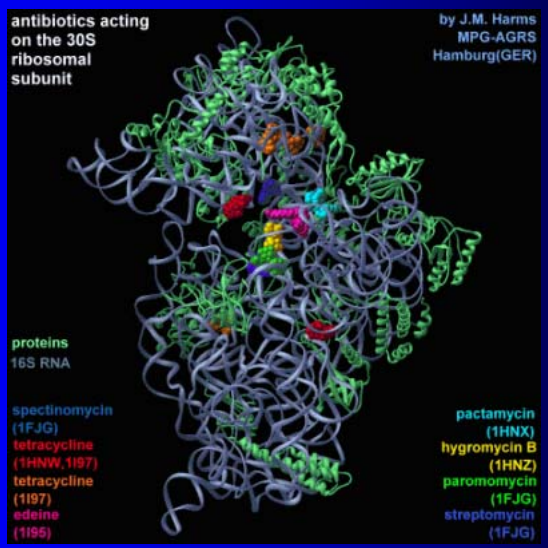


Bacterial Protein Synthesis - 70S Ribosome

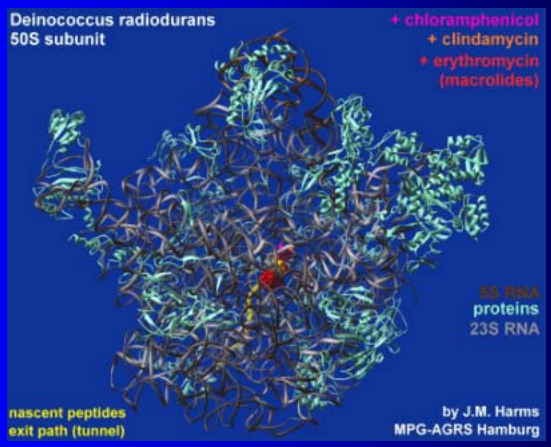


1) Charged tRNA carries amino acid
2) Binds to acceptor site on 50S subunit
3) Peptidyl tRNA already on donor site on 50S subunit
4) Peptidyl transferase catalyzes donation of growing peptide chain to aminoacyl tRNA
5) tRNA at donor site released (uncharged)
6) Growing chain at acceptor site moves to donor site and process starts again

Blocking the 30S Ribosomal Sub-unit 1



Blocking the 50S Ribosomal Sub-unit 2

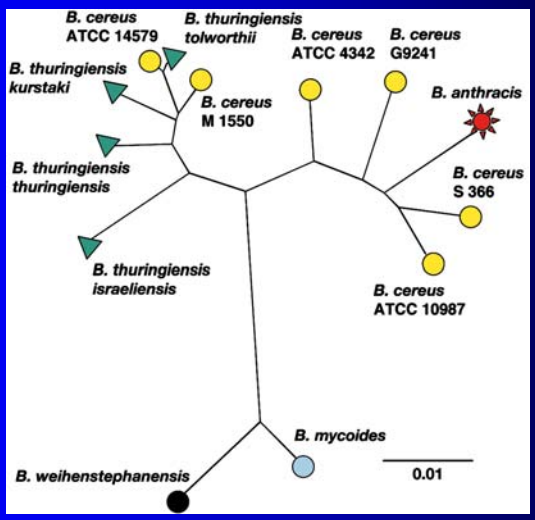


Bacteriostatic Antibiotics binding to 50S in Peptidyl Tranferase Center (Clindy), or in protein 'exit tunnel' (Azithromycin)

Plasmids Make for a Toxic 'Pea-Soup'

- 367 Microbial Genomes have now been sequenced, and another 619 have been partially completed.
- It has become obvious that most Bacterial species are not homogenous, consisting not only of a single chromosome, but also possessing a variable number of self-replicating plasmids carrying DNA, and Genes.
- *Borrelia burgdorferi* has a large number of plasmids, indeed, nearly half its genome exists on its 21 self-replicating plasmid sub-units
- Yet even common species, like *Staphylococcus epidermidis* ATCC 12228, give rise to plasmids (*S. Epidermidis* has 10% of its DNA spread over six self-replicating plasmids)
- These plasmids are not targeted by antibiotics, and, unless destroyed by the immune system, will persist in chronic intra-cellular infections – think of it as a 'DNA Pea-Soup'

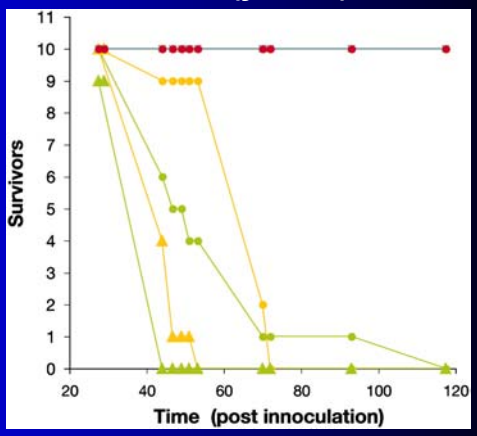
Plasmids transmit DNA 'horizontally'



Identification of anthrax toxin genes in a *Bacillus cereus* associated with an illness resembling inhalation anthrax
Hoffmaster, Alex R. et al. (2004) Proc. Natl. Acad. Sci. USA 101, 8449-8454

Plasmids transmit DNA 'horizontally'

Fig. 4. Survival of mice i.p. challenged with *B. cereus* G9241 hybrid (green), *B. anthracis Sterne* (yellow), and *B.cereus* ATCC10987 (red)



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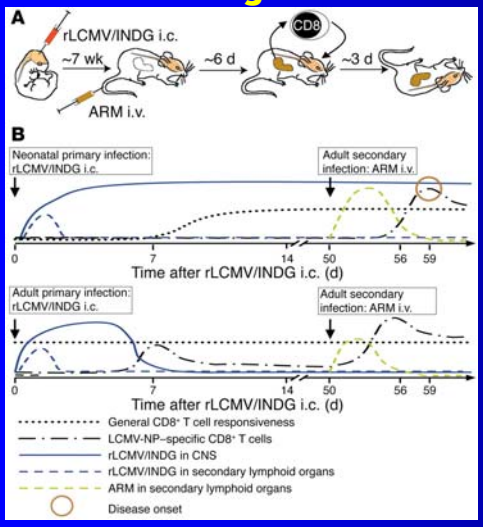
Rolf Zinkernagel Shows Pathogens persist in Brain



Merkler D, Zinkernagel R, et al.
J. Clin. Invest. 2006;116:1254-1263
Lymphocytic choriomeningitis virus,
Virus-specific cytotoxic T cells

- Because the innate CD8+ T-cell immune response takes 7 days (approx) to build after birth, LCMV infection persists in CNS only if the primary infection is neonatal. It is cleared from the CNS if the infection is first seen as an adult.
- This work is slowly dispelling the myth of 'Autoimmunity' or 'response to self' by showing that an occult virus can indeed persist in the CNS.

Question Time