Presentation 7 - Mohan Sopori

Immunotoxicity of low-dose sarin and silica inhalation

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Gulf War Syndrome (GWS)

- Some Gulf War veterans experience symptoms such as mood changes, loss of concentration, chronic fatigue, sleep disturbances, muscle and joint pains, skin rashes, Chronic digestive problems, loss of sex drive.
- Etiology of GWS is unknown. The war-related psychological stress, and exposures to nerve gas, anti-nerve gas prophylactics, insecticides, pathogens, vaccines, silica, etc have been considered to contribute to the symptomatology of the syndrome.

• EFFECTS OF SARIN AND SILICA ON THE IMMUNE SYSTEM

Potential immunological bases of the GWS

- Proinflammatory cytokines such as IL-1, IL-6, and TNF-α play a pivotal role in inflammation and neuroimmune-endocrine interactions.
- These cytokines ("alarm cytokines") are also produced in response to psychological stressors, and administration of high doses induces symptoms similar to those experienced by Gulf War veterans, i.e., arthralgia, headache, skin rash, fevers, decreased appetite appetite and libido, mood alterations, and fatigue.
- Agents such as sarin and silica might modulate the immune and inflammatory systems, leading to cytokine imbalances.

Nerve Gas (sarin) and Gulf war syndrome

- Sarin is a power nerve agent that in high doses may cause seizures and death.
- Its lethality and low cost of production makes it the chemical of choice for terrorism. The 1994 subway sarin attack in Japan, caused many deaths and injured over 6,000 people. Some of the survivors succumbed to Legionella infection.

Cholinergic Toxicity

- Inhibition of acetylcholinesterase (ChE) with organophosphates such as the nerve agent sarin, results in overabundance of acetylcholine (ACh) at the synaptic cleft, which might lead to lethality and convulsive seizures. Moreover, ChE inhibitors may induce psychopathologies that resemble the post-traumatic stress disorder.
- ACh stimulates both muscarinic and nicotinic receptors, and the lethality of anti-cholinesterase toxicity is reduced by cholinergic receptor antagonists together with the ganglionic blockers (e.g., chlorisondamine, hexamethonium), which act on the nicotinic receptors in the autonomic nervous system.
- Does sarin affect the immune system as nicotine?

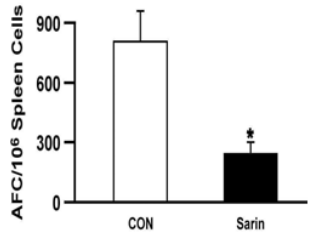
In rats and mice, chronic administration of nicotine suppresses the immune system, and the immunosuppression persists for several weeks, even when nicotine treatment is discontinued.

AFC/10^6 Spleen Cells

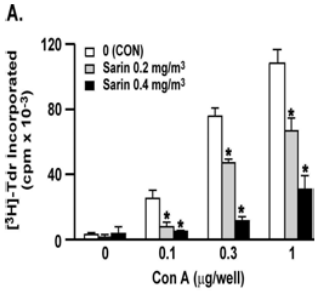
Treatment	n	Antibody-forming cells
Control	5	762 ± 106
Nicotine (4 wk)	4	296 ± 54
Post-nicotine (2 wk)	6	234 ± 70

Animals were implanted subcutaneously (s.c.) with saline (control)- or NT-containing miniosmotic pumps, and 4 days prior to sacrifice, animals were immunized with SRBC. Spleen cells were analyzed for anti-SRBC AFC responses by standard methods (Sopori et al., 1989).

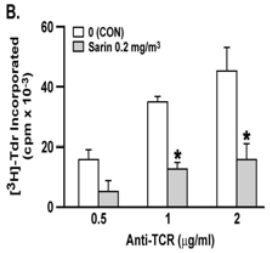
Sarin inhibits antibody response



Sarin suppresses T cell mitogenesis

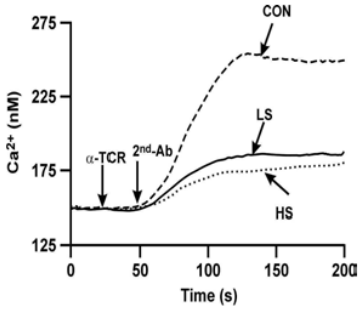


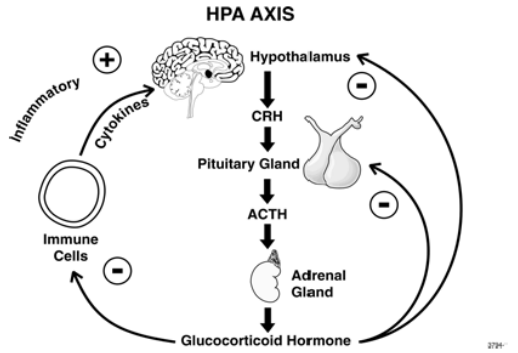
Sarin inhibits antigen-induced T cell proliferation

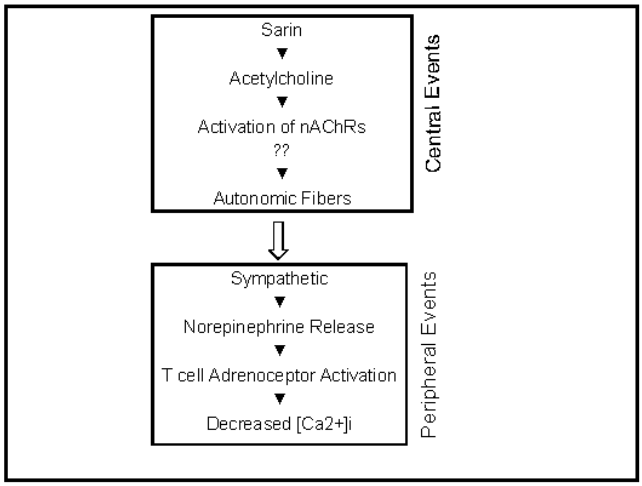


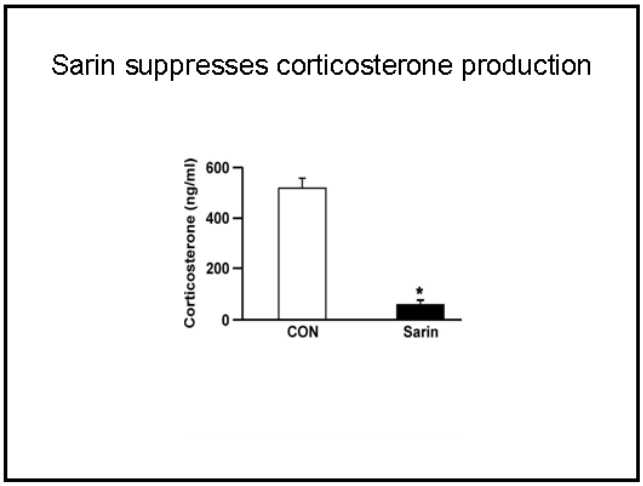
TCR + Ag
↓
Src-like PTK (Fyn)
↓
PTK
↓
Phospholipase C-γ1 (PLC-γ1)
↓
Inositol-1,4,5-trisphosphate (IP3)
↓
Increased $[Ca^{2+}]_i$
↓
G0/G1 → S-phase

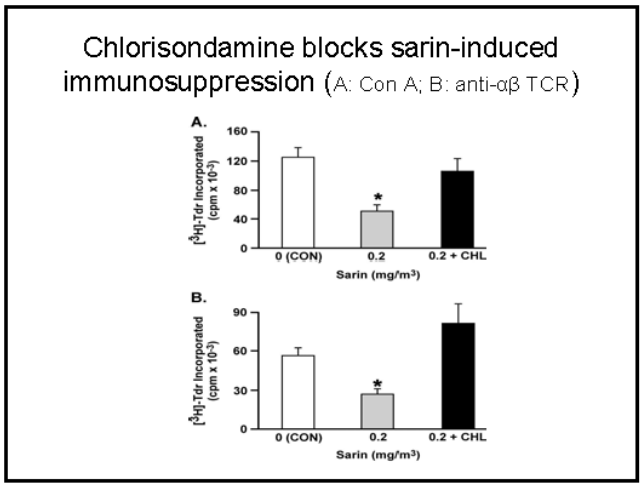
Sarin impairs T cell signaling

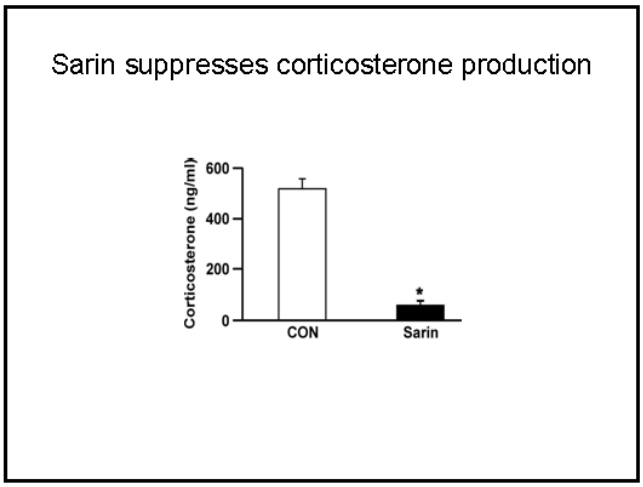




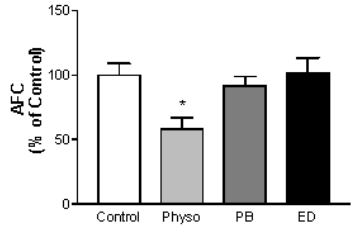

Sarin
Acetylcholine
Activation of nAChRs
??
Autonomic Fibers
Central Events
Sympathetic
Norepinephrine Release
T cell Adrenoceptor Activation
Decreased [Ca2+]i
Peripheral Events


Sarin suppresses corticosterone production
Corticosterone (ng/ml)
600
400
200
0
CON
Sarin

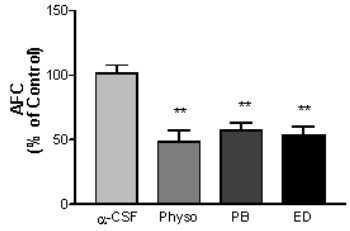

Chlorisondamine blocks sarin-induced immunosuppression (A: Con A; B: anti-αβ TCR)
A.
[3H]-Tdr Incorporated (cpm x 10-3)
160
120
80
40
0
0 (CON)
0.2
0.2 + CHL
Sarin (mg/m3)
B.
[3H]-Tdr Incorporated (cpm x 10-3)
90
60
30
0
0 (CON)
0.2
0.2 + CHL
Sarin (mg/m3)


Sarin suppresses corticosterone production
Corticosterone (ng/ml)
600
400
200
0
CON
Sarin

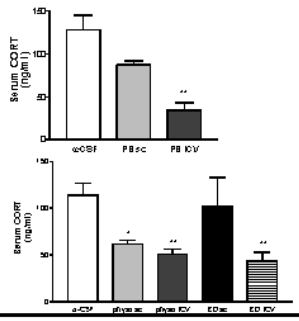
SC administration of physostigmine (0.2 mg/kg), but not pyridostigmine (0.5 mg/kg) or edrophonium (1.5 mg/kg) inhibits the antibody response



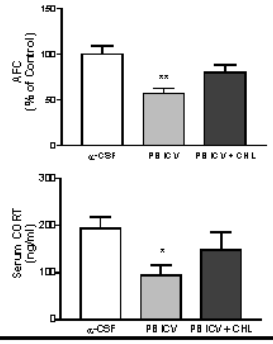
Intracerebroventricular administration of low doses of physostigmine (0.02 mg/kg), pyridostigmine (0.5 mg /kg), or edrophonium (1.5 mg/kg) inhibits the antibody response

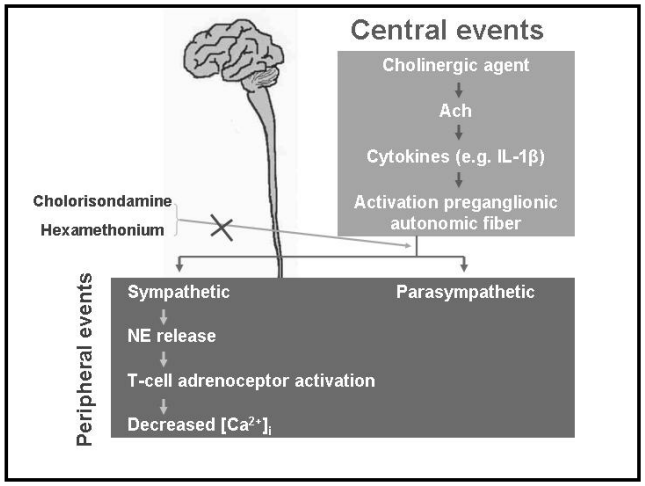


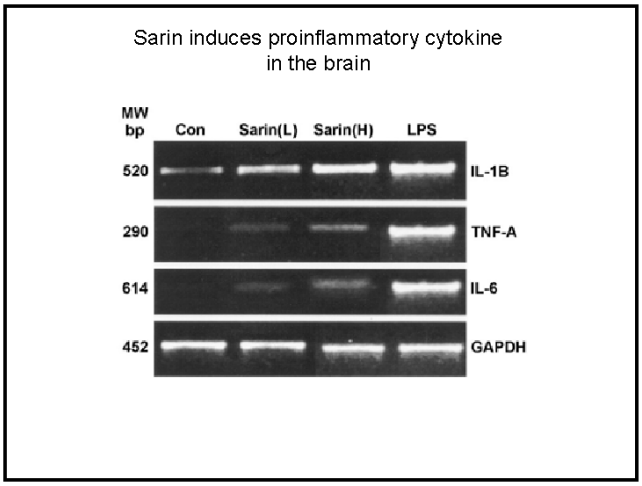
Central administration of cholinergic agents suppresses glucocorticoid production

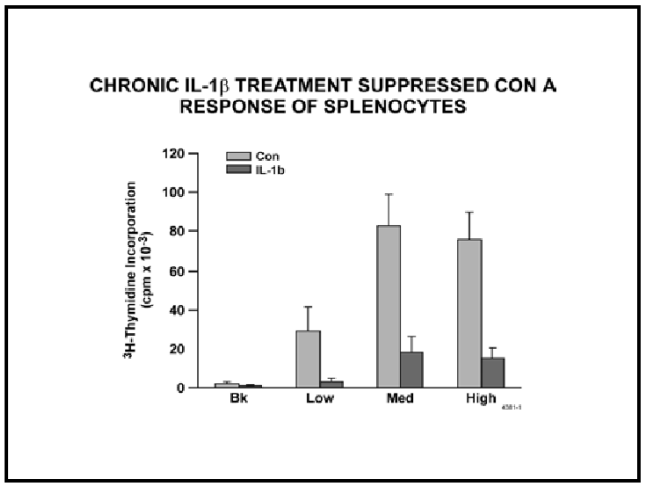


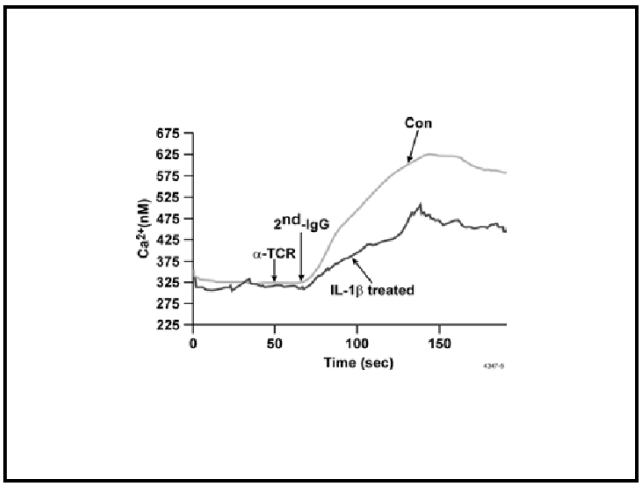
The ganglionic blocker, chlorisondamine (CHL), attenuates the inhibitory effects of pyridostigmine on the antibody and glucocorticoid responses

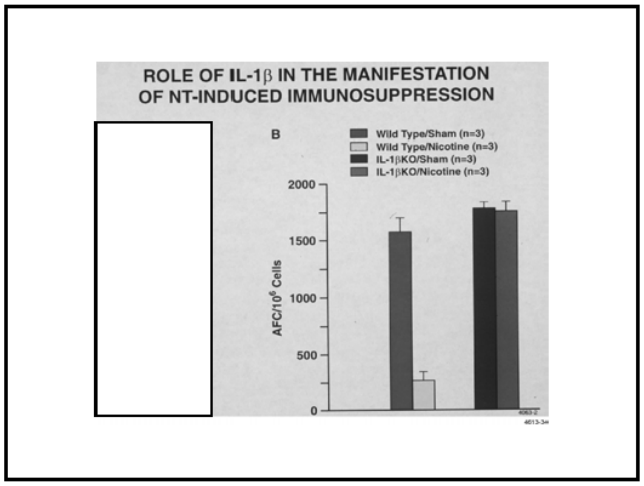


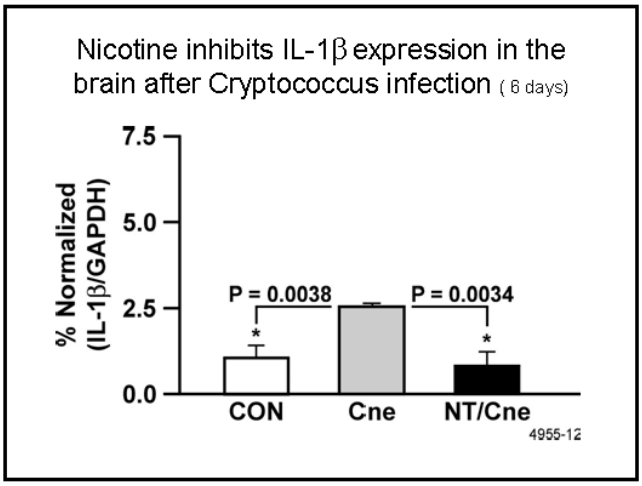

Central events
Cholinergic agent
Ach
Cytokines (e.g. IL-1β)
Activation preganglionic autonomic fiber
Cholorisondamine
Hexamethonium
Peripheral events
Sympathetic
Parasympathetic
NE release
T-cell adrenoceptor activation
Decreased [Ca2+]i

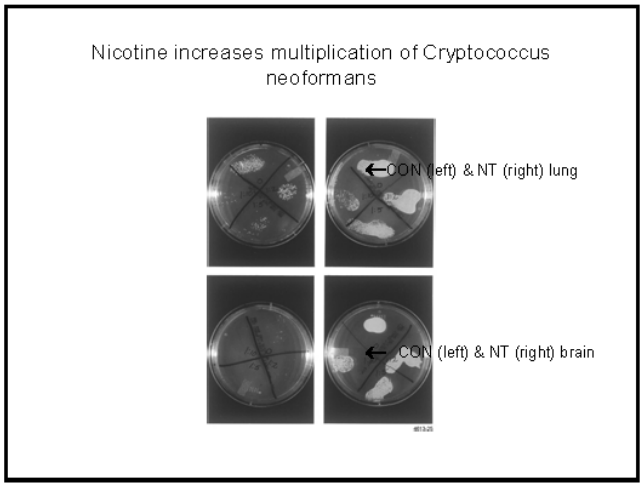

Sarin induces proinflammatory cytokine in the brain
MW bp
Con
Sarin(L)
Sarin(H)
LPS
520
IL-1B
290
TNF-A
614
IL-6
452
GAPDH

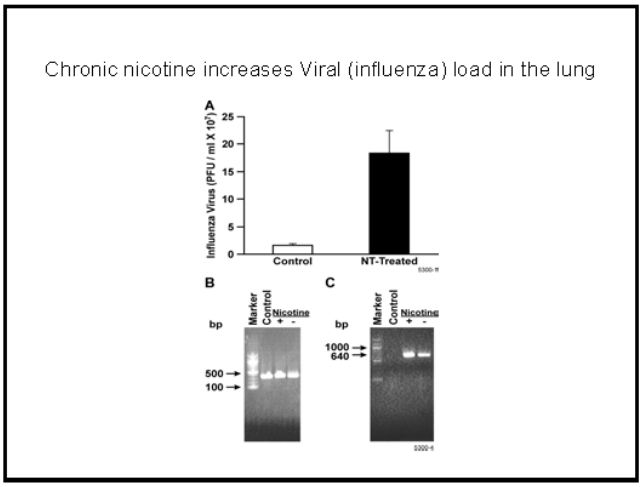

CHRONIC IL-1β TREATMENT SUPPRESSED CON A RESPONSE OF SPLENOCYTES
Con
IL-1b
3H-Thymidine Incorporation (cpm x 10-3)
120
100
80
60
40
20
0
Bk
Low
Med
High


Con
675
625
575
525
475
425
375
325
275
225
Ca2+(nM)
2nd-IgG
α-TCR
IL-1β treated
0
50
100
150
Time (sec)


ROLE OF IL-1β IN THE MANIFESTATION
OF NT-INDUCED IMMUNOSUPPRESSION
B
Wild Type/Sham (n=3)
Wild Type/Nicotine (n=3)
IL-1βKO/Sham (n=3)
IL-1βKO/Nicotine (n=3)
AFC/10^6 Cells
2000
1500
1000
500
0


Nicotine inhibits IL-1β expression in the
brain after Cryptococcus infection (6 days)
% Normalized
(IL-1β/GAPDH)
7.5
5.0
2.5
0.0
P = 0.0038
P = 0.0034
*
*
CON
Cne
NT/Cne
4955-12


Nicotine increases multiplication of Cryptococcus
neoformans
CON (left) & NT (right) lung
CON (left) & NT (right) brain


Chronic nicotine increases Viral (influenza) load in the lung
A
Influenza Virus (PFU / ml X 10^3)
25
20
15
10
5
0
Control
NT-Treated
B
C
bp
Marker
Control
Nicotine
+
-
500
100
1000
640

CONCLUSIONS

- Sarin and other cholinergic agents suppress the immune system.
- Cholinergic agents affect the immune system centrally through the autonomic nervous system.
- Sarin induces IL-β in the brain, and Chronic ICV administration of IL-1β suppresses the immune system. This immunosuppression may increase the risk for infections.
- Sarin and other cholinergic agents inhibit glucocorticoid production primarily through the autonomic nervous system. Increased plasma cortisol levels might serve as a marker for cholinergic exposure.

SILICA AS AN IMMUNOMODULATOR

- Saudi Arabian sand is rich in silica and contains a significant fraction of ultra-fine particles and microbial material.
- Silica is an adjuvant and, in susceptible individuals, might induce lung granulomas and autoimmune disorders.

SILICOSIS

- Silicosis is usually associated with occupational exposure to crystalline silica.
- Silicosis is usually diagnosed many years after the SL exposure; thus, the disease might progress over many years after the exposure has ended.
- In most experimental silicosis, animals show rapid lung inflammation and injury.

Methods

- Most prevalent animal models of silicosis use IT administration of large silica doses, leading to acute lung injury (apoptosis).
- Rats were exposed to silica inhalation (5-6 mg/m3, 6 h/d, 5 d/wk for 6 wk).
- We determined the immunological and inflammatory status of these animals.
- **CONCLUSION:** Acute silicosis may be mechanistically different from chronic silicosis.

Tissue silica burden (ng/mg) at the end (0 time) and 10 wk after silica treatment¶

Time§	Lung	Spleen	Brain	Liver
0 Time				
SL	413 ± 59	708 ± 154	186 ± 48	ND
10 Weeks				
SL	111 ± 8	51 ± 3	38 ± 3	50 ± 13

[a] SL, silica-treated; ND, not done.
[b] Represents time after SL exposure.
[c] Values represent mean ± SEM from five animals/group.

Histopathological changes in the lung and BALT at various times after silica exposure¶

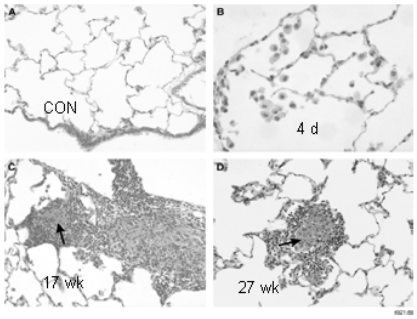
	Lung		
Sacrifice time	Focal AM§	Alveolar PMN/LYMP§	Granuloma
Day 4	0.4	0	0
4 wk	0.8	0	0
10 wk	1.4	1.6	0
17 wk	3.0	2.8	0.4
27 wk	2.5	2.5	2.0

¶Numerical values represent average grade from five animals/group.

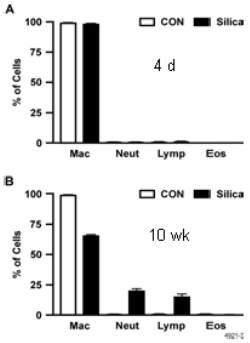
Silica increases protein and LDH content of BALF at later stages

Time§	LDH (U/L)	Protein (mg/DL)
4 days		
CON	38.0 ± 4.0[c]	2.0 ± 0.3
SL	44.0 ± 3.0	2.3 ± 0.2
10 weeks		
CON	42.0 ± 3.5	2.2 ± 0.4
SL	47.0 ± 4.0	2.8 ± 0.5
17 weeks		
CON	23.4 ± 2.0	2.4 ± 0.4
SL	100.6 ± 24.0 *	6.4 ± 0.7 **

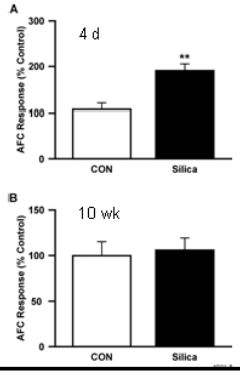
* P ≤ 0.05; ** P ≤ 0.005.



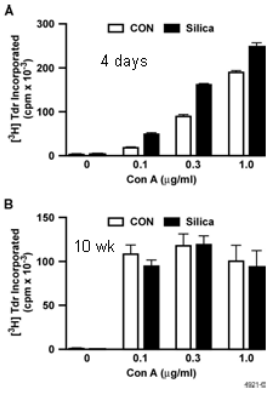
Accumulation of leukocytes in the lung occurs in the later phase of chronic silicosis



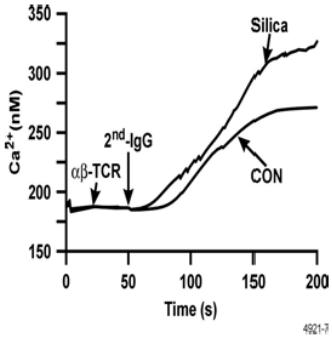
Silica activates antibody response in the early phase of silicosis

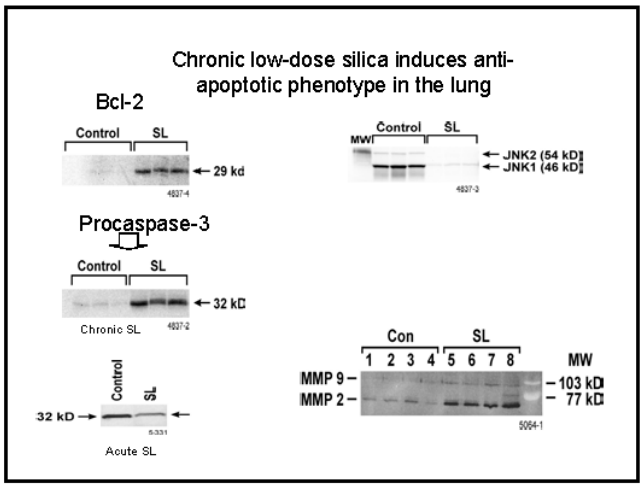


Silica stimulates Con A response in the early phase



Low-dose chronic silica stimulates the antigen-induced rise in the intracellular calcium level in T cells in the early phase of silicosis





CONCLUSION

- In susceptible individuals, amount of silica required to induce silicosis may be small.
- Unlike acute silicosis, chronic silicosis shows a delayed inflammatory response and the granuloma formation is associated with anti-apoptotic phenotype.
- At present, the mechanism and the biological consequences of silica accumulation in the brain is unclear.

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