

I. Leykin · B. Spivak · A. Weizman · I. R. Cohen
M. Shinitzky

Elevated Cellular Immune Response to Human Heat-Shock Protein-60 in Schizophrenic Patients

Received: 26 November 1998 / Accepted: 10 July 1999

Abstract Heat shock protein-60 (HSP60) is implicated in several autoimmune diseases as a triggering antigen. Based on the autoimmune hypothesis of schizophrenia, we examined cellular and humoral responses against HSP60 and a series of its peptide fragments with peripheral blood samples of schizophrenic patients and healthy subjects each of group size between 12 to 32 participants. The average stimulation indices of peripheral blood mononuclear cells (PBMC) to HSP60 were 3.17 ± 0.36 (mean \pm SE) for schizophrenic patients and 2.23 ± 0.24 (mean \pm SE) for healthy subjects, with a significant difference between the groups ($P = 0.0457$). In parallel, 38 synthetic peptide fragments of HSP60, each of 18–21 amino acids, were tested for in vitro sensitization of PBMC. With one peptide (p32) the average stimulation index of PBMC from schizophrenic patients was significantly higher than that obtained for PBMC of control subjects ($P = 0.0006$). Comparing the cellular immune response to p32 between patients who were distinctive responders ($n = 10$) or non-responders ($n = 10$) to neuroleptic treatment indicated a similar elevation of cellular response in these groups. Antibodies against HSP60 were screened by dot-blot and ELISA in the sera of the above blood samples. Titers of IgG and IgM against HSP60 were found to be of similar magnitude in schizophrenic

patients and in controls. Titers of IgA against HSP60 were somewhat higher in the sera of schizophrenic patients in comparison to sera of control subjects ($P = 0.0605$).

Key words Autoimmunity · autoantigen · heat shock protein-60 · immune response · schizophrenia

Introduction

A series of immunological abnormalities have been ascribed to schizophrenia. They include elevated serum immunoglobulins (Sugerman et al. 1982, DeLisi et al. 1985), decreased mitogenic response of peripheral blood lymphocytes (Vartanyan et al. 1978, Ganguli et al. 1987, Chengappa et al. 1995), the presence of morphologically abnormal large lymphocytes in the blood and bone marrow (Vartanyan et al. 1978), increased serum IL-2 receptor levels (Ganguli and Rabin 1989, Ganguli et al. 1989, Rapaport et al. 1989, Wilke et al. 1996), decreased IL-2 (Ganguli et al. 1989, Villemain et al. 1989, Ganguli et al. 1992, Ganguli et al. 1995, McAllister et al. 1995) and IFN- γ production (Rothermundt et al. 1996, Wilke et al. 1996), and a high serum level of IL-6 (Shintani et al. 1991). With respect to lymphocyte sub-populations, Nyland et al. (1980) found a reduced percentage of T cells in schizophrenic patients with acute relapse. Masserini et al. (1990) found an increase of T suppressor lymphocytes in drug-free schizophrenic patients, while the neuroleptic-treated patients showed an increase of T helper lymphocytes. A decrease in the percentage of T cells in schizophrenic patients during acute attack and an increase in the helper/suppressor T cell ratio were found to correlate with the psychiatric status (Coffey et al. 1983).

As a whole, these findings point to an aberration in the immune function of schizophrenic patients, which could promote an autoimmune reaction and, in turn, might contribute to the psychotic state (Noy et al. 1994). The presence of antibody autoantibodies in schizophrenic patients (Heath et al. 1989, Knight et al. 1990, Henneberg et al. 1994, Yang et al. 1994), which can induce behavioral

Acknowledgement This study was supported by a grant from the Mayer Family Foundation

Prof. M. Shinitzky (✉) · I. Leykin
Department of Biological Chemistry,
Weizmann Institute of Science, Rehovot, 76100, Israel
e-mail: BMSHINIT@WEIZMANN.WEIZMANN.AC.IL

B. Spivak
Ness-Ziona Psychiatric Hospital, Ness-Ziona, Israel

A. Weizman
Geha Psychiatric Hospital, Petach-Tikva, Israel

I. R. Cohen
Department of Immunology,
Weizmann Institute of Science, Rehovot, 76100, Israel

changes upon injection to animals (Pandey et al. 1981), supports this possibility. It was therefore proposed that the pathogenesis of schizophrenia is associated to some extent with autoimmune elements (Knight 1984, Wright and Murray 1993, Noy et al. 1994, Wright et al. 1996). Indeed, schizophrenia shares several features with recognized autoimmune diseases such as Graves' disease, insulin-dependent diabetes mellitus (IDDM), and rheumatoid arthritis (RA) (Knight 1984). Approximately 50% concordance in identical twins was also found in schizophrenia (Knight and Adams 1982), as well as an association between histocompatibility antigens and disease susceptibility, with an increase in the frequency of HLA A2, A23 (Amar et al. 1988), A10, A11, A29 (Oscan et al. 1996), B7 (Sorokina et al. 1987), and DR2 (Dvorakova et al. 1989).

Indirect support for the autoimmune hypothesis of schizophrenia is reflected in co-morbidity correlations, which are typical to autoimmune diseases, and seem to apply to schizophrenia, as well. Finney (1989) reported a negative correlation between IDDM and schizophrenia, while in schizophrenic patients the incidence of IDDM is much less than in the normal population. A similar negative epidemiological correlation was also observed for schizophrenia and RA (Mellsop et al. 1974, Ostenberg 1978, Allebeck et al. 1985, Malck-Ahmadi 1985, Spector and Silman 1987, Spector and Silman 1990, Vinogradov et al. 1991, Eaton et al. 1992). Overall, schizophrenic patients have a four- to six-fold reduction in the risk of developing RA, and even a lower risk for IDDM. Another indirect evidence for the assertion of an autoimmune arm in schizophrenia comes from our recent reports on psychiatric improvements in schizophrenic patients treated with the immunosuppressive drug azathioprine (Levine et al. 1994, Levine et al. 1997).

Autoimmunity to heat shock proteins (HSPs) seems to be involved in the etiology of RA (van Eden et al. 1988, De Graeff-Meeder et al. 1991, Hermann et al. 1991, Life et al. 1991), IDDM (Cohen 1991, Elias et al. 1991, Birk et al. 1996, Maclaren and Atkinson 1997), Behçet's disease (Pervin et al. 1993, Hasan et al. 1996), and probably in other autoimmune diseases, like multiple sclerosis, atherosclerosis, systemic sclerosis, systemic lupus erythematosus, and Graves' disease (Rajagopalan et al. 1990, Selmaj et al. 1991, Danieli et al. 1992a, Heufelder et al. 1992, Xu et al. 1992). However, only scarce information is currently available on the involvement of HSP induced autoimmunity in schizophrenia. A possible implication of HSP autoimmunity in schizophrenia could be inferred from the work of Kilidireas et al. (1992), who reported an increase of antibodies against HSP60 in schizophrenic patients. Recently, Schwarz et al. (1998) reported on elevated anti-HSP60 antibodies in subgroups of 10–20% of schizophrenic patients, which correlated with high levels of serum IL-2 and ICAM-1, as well as with increased blood-brain permeability. Similarly, Mazeh et al. (1998) have detected antibodies to HSP85 in the serum of schizophrenic patients.

Heat shock proteins are a family of conserved proteins which are synthesized in response to elevation of temper-

ature and other types of physiological stress (Jindal et al. 1989, Yound 1990). Their precise function is not fully understood, but they seem to play an important role as chaperons (Srivastava 1994). Bacterial HSPs are major immune targets in a broad spectrum of infectious diseases (Young et al. 1988, Kaufmann 1990, DeNagel and Pierce 1993) and have extremely close homologues in mammalian cells (Dudani and Gupta 1989, Winfield 1989). A recent review (Kaufmann 1994) summarizes the evidence for the possibility that HSP molecules may act as triggering targets in autoimmune diseases.

If indeed HSPs were to be involved in the pathogenesis of schizophrenia, then it might be possible to detect their corresponding humoral or cellular responses, which were examined in this study.

Materials and methods

Patients and control subjects

Thirty two schizophrenic patients of various categories and stages participated in the study. Demographic distribution of the participating patients is given in Table 1. All the patients were free of acute or chronic disorders which could affect immune function, and their laboratory blood tests, including CRP and rate of sedimentation, were in the normal range. The PANSS psychiatric rating (Kay et al. 1990) was carried out by an independent group of psychiatrists on the day of blood drawing.

Healthy volunteers, mostly of the hospital staff, constituted the control group. These were 14 males and 7 females, age 27–61 (39.7 ± 11.1 years; mean \pm SD).

Antibodies and antigens

Recombinant human HSP60 was a β -galactosidase fusion construct expressed from plasmid pRH710 (Boog et al. 1992). Overlapping peptide fragments of human HSP60 p1-p38, each of 18–21 amino acids, were synthesized and purified as described (Elias et al. 1991). Recombinant human HSP27, HSP70, HSP90, recombinant *E. coli* DnaJ, GrpE, GroEL, GroES, and DnaK were supplied by StressGen Biotechnologies Corp. (Victoria, Canada). Tetanus Toxoid (TT) was purchased from RIVM (Netherlands).

Peroxidase-conjugated AffiniPure Goat Anti-Human IgG (Fc), Alkaline Phosphatase-conjugated AffiniPure Goat Anti-Human IgM (Fc) and Streptavidin-Peroxidase conjugate were obtained from Jackson ImmunoResearch Laboratories, Inc. (PA, USA). Alkaline Phosphatase-conjugated Mouse Anti-Human IgA₁/A₂ mAb was supplied by Pharmingen (USA). Alkaline Phosphatase-conjugated Rabbit Anti-Human IgE was purchased from DAKO (Denmark). Biotin Mouse Monoclonal Anti-Human IgG1, IgG2, IgG3, and

Table 1 Demographic data of the schizophrenia patients of this study

Gender and treatment	n	age range
Females	13	26–61
Males	19	23–56
Under drug treatment*	28	
Responders	22	
Weakly or non-responders	10	
Untreated or newly diagnosed	4	

* Conventional neuroleptics, except for clozapine

IgG4 were obtained from Sigma Chemical Co. (USA). Mouse Anti-Human IFN- γ mAb and Biotinylated Anti-Human IFN- γ mAb were supplied from Genzyme Diagnostics (USA). Human rIFN- γ was purchased from Pharmingen (USA).

Peripheral blood mononuclear cells (PBMC)

Human peripheral blood samples, 10–12 ml with heparin anti-coagulant, were drawn in the morning and processed within 4 hours. Platelet rich plasma was collected by slow centrifugation (100 g for 20 min. at room temperature) and discarded. The collected blood cells were suspended in 40 ml of HBSS and layered gently on 10 ml Ficoll-Paque (Pharmacia, Sweden) and centrifugated at 900 g for 20 min. at room temperature. The interface layer of peripheral blood mononuclear cells (PBMC), composed predominantly of lymphocytes, was collected, and contained $7-9 \times 10^6$ cells.

In vitro stimulation of PBMC

Tests were carried out in 96 microwell plates (Nunk, Denmark) in triplicate in a humidified atmosphere with 5% CO₂ at 37°C. Each well contained 200,000 PBMC suspended in 200 μ l of RPMI 1640 medium supplemented with 2% heat inactivated human AB serum, 2 mM L-glutamine, penicillin (100 units/ml), streptomycin (100 μ g/ml), gentamycin sulfate (50 μ g/ml), 1% non-essential amino acids, 1 mM sodium pyruvate, 5×10^{-5} M β -mercaptoethanol, and 20 mM HEPES, pH 7.2. Antigens were added at a final concentration of 10 μ g/ml for HSP60 and 25 μ g/ml for the peptide fragments of HSP60. Wells with Tetanus Toxoid (TT) (1 μ g/ml) served as positive controls. Wells without antigens or mitogens were used as negative controls.

Stimulation of PBMC was scored after 7 days by pulsing with 1 μ Ci per well of ³H-thymidine (Amersham, UK) for the last 18 hours. Supernatants were collected and stored at -20°C and subsequently used for IFN- γ determination (see below).

The stimulation index (SI) was defined as the ratio of mean cpm of test cultures divided by the mean cpm of control cultures without antigen.

IFN- γ determination

IFN- γ secretion was measured by enzyme immunoassay (Abrams et al. 1992). Microtiter plates (Nunk, Denmark) were coated with purified mouse antihuman IFN- γ monoclonal antibodies by over-

night incubation at 4°C. After washing with phosphate buffered saline (PBS)/0.05% Tween 20, the wells were blocked with PBS/10% fetal calf serum for 2 hours at room temperature. Standards and supernatants from the cell cultures were added to the washed wells and incubated for 4 hours at room temperature. Then biotinylated anti-IFN- γ detecting mAb were added. After an additional incubation for 45 min. at room temperature and washing, the streptavidin-peroxidase conjugate was added and incubated for 30 min at room temperature and then washed 4 times. A mixture of H₂O₂ and 2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (Sigma Chemical Co., USA) was added. The color was developed at room temperature (10–80 min.) and stopped by adding SDS/N,N-dimethyl formamide (Sigma Chemical Co., USA). OD values were measured at 405 nm and evaluated with the aid of a calibration curve. Values of spontaneous IFN- γ secretion, usually below the detection limit, were subtracted from the overall reading.

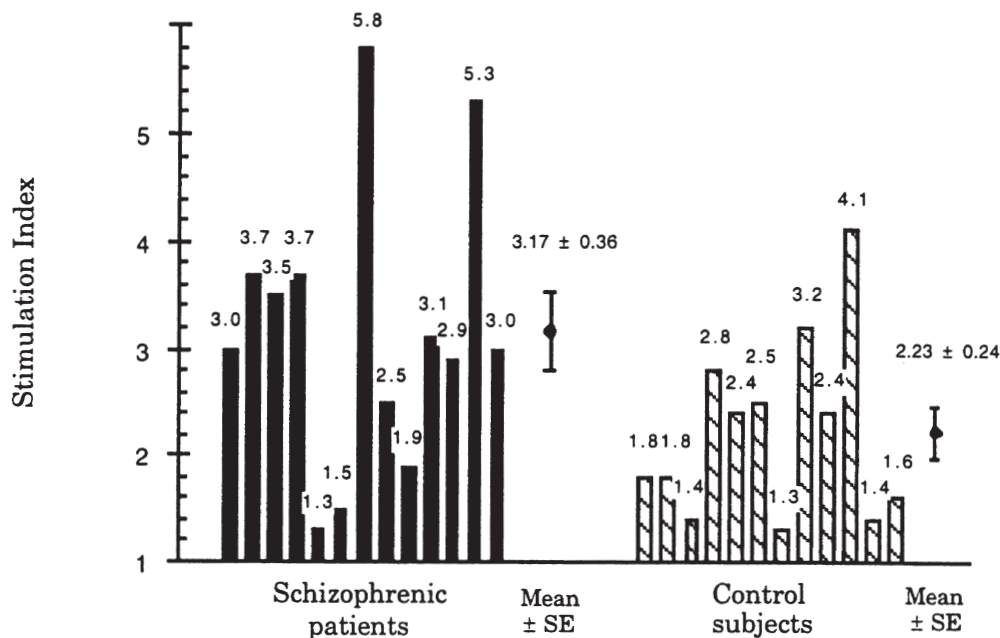
Dot-blot experiments

One microliter of different concentrations of HSP (1 μ g/ μ l, 100 ng/ μ l, 10 ng/ μ l, 1 ng/ μ l, 100 pg/ μ l, 10 pg/ μ l, and 1 pg/ μ l), human (HSP27, HSP60, HSP70, HSP90) or bacterial (DnaJ, GrpE, GroEL, GroES, DnaK), diluted in PBS, were applied onto nitrocellulose. The spots were dried and then probed with 200 μ l of sera from schizophrenic or control subjects by overnight incubation at room temperature, with gentle shaking in 800 μ l pH 7.7 buffer containing 60 mM citric acid, 90 mM disodium phosphate, 200 mM sodium chloride, 168 mM sodium hydroxide, and 0.4% Tween 20. After 3 washings with the same buffer, positive reactions were detected by HRP goat anti-human IgG-Fc Ab at a final dilution of 1:5000 in 200 mM Tris, 200 mM KCl, 0.1% Triton X-100, 10.5 mM phenol, and 2.1 mM CaCl₂, pH 8.0, for 4 hours at room temperature with 3 mg/ml 4-chloronaphthol (Sigma Chemical Co., USA) in methanol containing 3% H₂O₂ as the color releasing substrate.

Detection of anti-HSP60 antibodies

Human HSP60 or TT (as a positive control antigen), diluted in PBS (10 μ g/ml), was adsorbed onto microtiterplates by 4 hours incubation at room temperature, followed by overnight incubation at 4°C. After removing unbound protein, the wells were blocked by incubation with PBS/1% BSA for 2 hours at room temperature. After 4 washings with PBS/0.05% Tween 20, sera from schizophrenic patients and control subjects, diluted 1/10, were added and incubated for 2 hours at 37°C. Following 4 washings with PBS/

Fig. 1 Stimulation of lymphocytes from schizophrenic patients (n = 13) and control subjects (n = 12) by human HSP60. The difference in the responses was significant (P = 0.0457)



0.05% Tween 20, peroxidase conjugated anti-human IgG (Fc) mAb (1/1000) was added and incubated for 2 hours at room temperature. After washing, a mixture of H₂O₂ and 2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (Sigma Chemical Co., USA) was added. The color reaction was developed at room temperature (10–80 min.) and stopped by adding SDS/N,N-dimethyl formamide (Sigma Chemical Co., USA). The plates were scored by OD measurement at 405 nm.

Statistical significance

The Mann-Whitney test (two-tailed P value) was used to evaluate significant differences between experimental and control groups.

Results

This study was carried out in separate segments where different numbers of subjects participated.

Cell-mediated response against HSP60 was tested by lymphocyte stimulation *in vitro*. The results are shown in Fig. 1. Taking a stimulation index of ≥ 3.0 as positive response, the cellular response against HSP60 was positive in most schizophrenic patients (62%) compared to only 17% in the control group (SI 3.17 ± 0.36 in schizophrenic patients and 2.23 ± 0.24 in controls, mean \pm SE; $P = 0.0457$).

The above findings prompted us to investigate whether specific peptides of HSP60 might activate lymphocyte responses, as has been found in other autoimmune diseases (Hunt et al. 1993, Anderton et al. 1995, Elias et al. 1995). Thirty-eight peptide fragments of human HSP60, each of 18–21 amino acids, were assayed for lymphocyte stimulation. Table 2 summarizes the results. One particular fragment (p32) acted as an efficient stimulant of PBMC from some schizophrenic patients while cells from healthy subjects responded significantly less to this peptide, (3.18 ± 0.38 in schizophrenic patients compared to 1.73 ± 0.10 in controls, mean \pm SE; $P = 0.0006$; see Fig. 2). An arbitrary upper normal value of SI ≈ 3 indicates that 17 out of the 32 patients have an abnormal response to p32 (see Fig. 2). We have further verified whether the spread in response of PBMC from schizophrenic patients to p32 (see Fig. 2) is empirically related to the degree of responsiveness to neuroleptic drugs. As shown in Fig. 3, PBMC from responders reacted significantly stronger to p32 than PBMC from control subjects ($P = 0.0147$). Non-responders showed a similar higher response as the responders with no significant difference between these groups ($P = 0.1655$).

Supernatants from cell cultures stimulated with HSP60 were assayed for IFN- γ . As shown in Fig. 4, IFN- γ secretion of lymphocytes from schizophrenic patients was significantly higher than in control samples (3530 ± 228 pg/ml in schizophrenic patients compared to 2702 ± 301 pg/ml in controls, mean \pm SE; $P = 0.0358$). No difference was found in IFN- γ secretion, as well as in lymphocyte stimulation, between patients treated or untreated with neuroleptics. Moreover, no correlation was found between the magnitude of ³H-thymidine incorporation and that of IFN- γ secretion in either the schizophrenic subjects or the controls (data not shown).

Table 2 Mean Stimulation Index of lymphocytes from schizophrenic patients (n = 8–12) by 25 μ g/ml of different fragments of HSP60. Indices higher than 2 are marked.

No.	Aminoacid sequence	Peptide fragment (residues)	Mean SI
1.	MLRLPTVFRQMRPVSRVLAP	1– 20	<u>2.5</u>
2.	RVLAPHLTRAYAKDVKFGAD	16– 35	1.0
3.	KFGADARALMLQGVDLLADA	31– 50	1.2
4.	LLADAVAVTMGPKGRTVIE	46– 65	1.3
5.	TVLIEQSWGSPKVTKDGVTV	61– 80	1.3
6.	DGVTVAKSIDLKDKYKNIGA	76– 95	1.3
7.	KNIGAKLVQDVANNTNEEAG	91–110	1.3
8.	NEEAGDGTTTATVLARSIK	106–125	1.1
9.	RSIAKEGFEKISKGANPVEI	121–140	1.0
10.	NPVEIRRGVMLAVDAVIAEL	136–155	1.2
11.	VIAELKKQSKPVTTPPEEIAQ	151–170	1.0
12.	EETAQVATISANGDKEIGNI	166–185	1.2
13.	EIGNIISDAMKKVGRKGI	181–199	1.1
14.	RKGVITVKDGKTLNDELEII	195–214	1.3
15.	ELEIEGKMFDRGYISPYFI	210–229	1.3
16.	SPYFINTSKGQKCEFDAYV	225–244	1.2
17.	QDAYVLLSEKKISSIQSIVP	240–259	1.5
18.	QSVPALEIANHRKPLVILIA	255–275	1.9
19.	LVIIEAEDVDGEALSTLVLNR	271–290	1.2
20.	LVLNRLKVGQLQVAVKAPGF	286–305	1.3
21.	KAPGFGDNRNQLKDMAIAT	301–320	1.1
22.	MAIATGGAVFGEEGLTLNLE	316–335	1.0
23.	TLNLEDVQPHDLGKVGVEIV	331–350	1.0
24.	GEVIVTKDDAMLLKGGDKA	346–365	1.2
25.	KGDKAQIEKRIQEIEQLDV	361–380	1.1
26.	EQLDVTTSEYEKEKLNERLA	376–395	1.0
27.	NERLAKLSDGVAVLKVGGTG	391–410	1.8
28.	VGGTSDVEVNEKKDRVTDAL	406–425	1.0
29.	VTDALNATRAAVEEGIVLGG	421–440	1.5
30.	IVLGGGCALLRCPALDSLIT	436–455	1.0
31.	LDSLTPANEDQKIGIEIKR	451–470	1.0
32.	EIKRTLKIPAMTIAKNAGV	466–485	<u>3.2</u>
33.	KNAGVEGSLIVEKIMQSSSE	481–500	1.0
34.	QSSSEVGYDAMAGDFVNMVE	496–515	1.3
35.	VNMVEKGIIDPTKVVRTALL	511–530	1.0
36.	RTALLDAAGVASLLTTAEVV	526–545	1.3
37.	TAEVVVTEIPKEEKDPGMGA	541–560	1.0
38.	PGMGAMGGMGGMGGGMF	556–573	1.3

Antibody binding to various HSP antigens was assayed using a dot-blot technique. Sera from schizophrenic patients, who were either treated with neuroleptics or free of treatment, as well as from normal controls, reacted only slightly with human HSP27, HSP70, HSP90, and E. coli GrpE, GroEL, GroES, DnaK, and DnaJ. Furthermore, no difference was detected between sera from schizophrenic patients and normal subjects, as well as between sera from schizophrenic patients under neuroleptic treatment or neuroleptic-free, in antibody titers to these antigens. Antibody titers against HSP60 are summarized in Table 3. The apparent difference between the IgG titers was actually insignificant and was due to exceptional high titers in 2

Fig. 2 Stimulation of lymphocytes from schizophrenic patients ($n = 32$) and control subjects ($n = 21$) by peptide p32 of HSP60 (see Table 1). The difference in response between the groups was highly significant ($P = 0.0006$). An arbitrary cutoff for the upper normal value was taken as $SI \approx 3$. Among the tested patients 53% scored SI above this cutoff

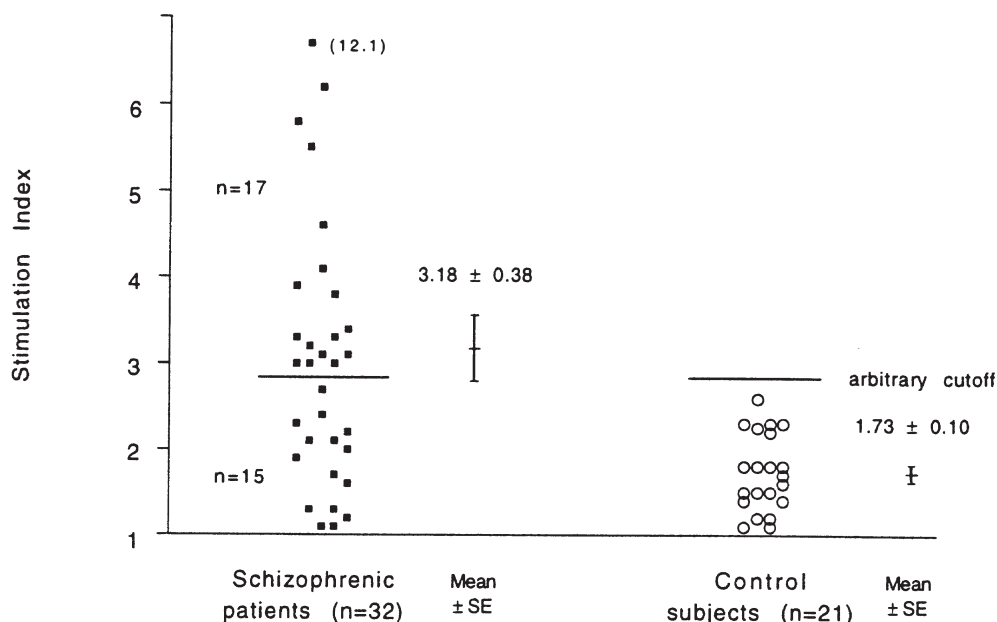
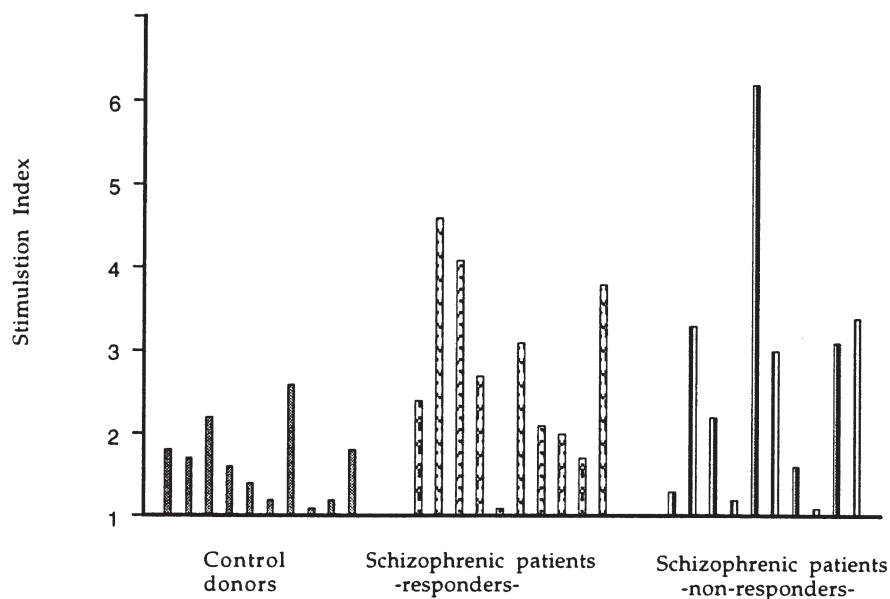


Fig. 3 Stimulation of lymphocytes from responders ($n = 10$) and non-responders ($n = 10$) schizophrenic and normal control subjects ($n = 10$) by peptide p32 of HSP60 (see Table 2). The difference in response between responders and control subjects was significant ($P = 0.0147$), while the difference between responders and non-responders was not significant ($P = 0.1655$)



cases in the control group. Subtypes of IgG in the humoral response against HSP60 were also assayed. No difference was found in the titers of IgG1 between schizophrenics and normal subjects. IgG3 and IgG4 titers were close to the limit of detection. The IgG2 subtype in schizophrenic sera was slightly lower in comparison to control sera; however, this difference was also not significant (data not shown).

The IgM response to HSP60 appeared to be slightly lower in schizophrenic patients (see Table 3), yet with only questionable significance.

Titers of IgA against HSP60 were found to be somewhat higher in schizophrenic patients in comparison to the control group. Titers of IgE against HSP60 were below the detection limit in both schizophrenic patients and healthy subjects (not shown).

In general our results on antibodies against HSP60 are in fair agreement with Schwarz et al. (1998) who observed that in 80–90% of the schizophrenic patients the titers fall in the normal range, while in remaining patients it is significantly elevated. It should be noted that no correlation was observed between the anti HSP antibody titers and the psychiatric evaluation of the individual patients. No correlation was found between the titers of IgG, IgM or IgA against HSP60 and ^3H -thymidine incorporation or IFN- γ release shown in Figs. 1–4. Moreover, there were no differences between the schizophrenic patients and the normal subjects in their antibody and cellular responses to TT, except for an IgA response to this antigen which was slightly higher in the schizophrenic patients (not shown).

Fig. 4 IFN- γ secretion of lymphocytes from schizophrenic patients and control subjects after stimulation by human HSP60. The difference in values between the groups was significant ($P = 0.0358$)

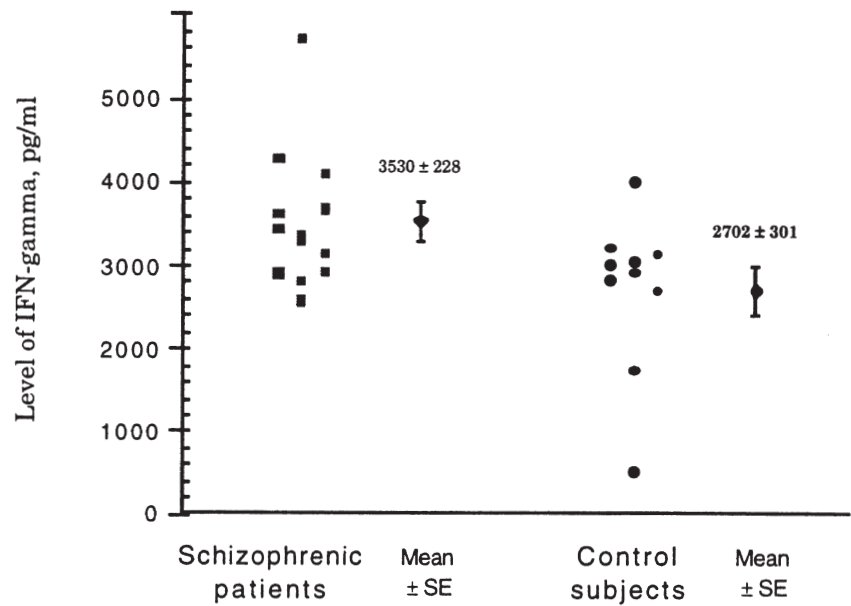


Table 3 Titers of antibodies against HSP60 in sera of schizophrenic patients ($n = 13$) and normal subjects ($n = 10$) presented as mean \pm S.E. of relative O.D. units

	IgG	IgM	IgA
Schizophrenic patients	0.043 \pm 0.012	0.254 \pm 0.030	0.122 \pm 0.027
Normal subjects	0.110 \pm 0.025 (2 cases with exceptionally high values)	0.373 \pm 0.054	0.047 \pm 0.020
Significance	$P = 0.150$	$P = 0.088$	$P = 0.060$

Discussion

The highly conserved sequence homology between HSPs of microbial and mammalian origins (Jindal et al. 1989) is now believed to underlie the etiology of some autoimmune responses (van Eden et al. 1988, Elias et al. 1990, Lamb and Young 1990, De Graeff-Meeder et al. 1991, Hermann et al. 1991, Res et al. 1991, Danieli et al. 1992b, Kaufmann 1994). Accordingly, part of the overt immune reaction to microbial infection may involve a T-cell response to the bacterial HSP that can then cross-react with self antigens, which might lead to an autoimmune response. Evidence from studies on RA (van Eden et al. 1988, De Graeff-Meeder et al. 1991, Hermann et al. 1991, Life 1991) and Behçet's disease (Pervin et al. 1993, Hasan et al. 1996) supports this concept. In IDDM, experimental evidence also suggests such an etiological pattern (Cohen 1991, Elias et al. 1991, Birk et al. 1996, Maclaren and Atkinson 1997). For other autoimmune diseases like multiple sclerosis, systemic lupus erythematosus, atherosclerosis, and Graves' disease, the evidence for the etiological involvement of HSPs is more indirect (Rajagopalan et al. 1990, Selmaj et al. 1991, Danieli et al. 1992, Heufelder et al. 1992, Xu et al. 1992).

It is reasonable to suppose that organ specific autoimmune diseases should involve some cross reactivity between a specific fragment of HSP60 and a homologous epitope on the attacked tissue (Cohen 1991). Specific peptide fragments of HSP60 seem to be targeted in IDDM (Elias et al. 1995), in RA (van Eden et al. 1991, Danieli et al. 1992b), and in Behçet's disease (Stanford et al. 1994, Direskeneli et al. 1996). The findings described here point to a specific fragment of HSP60 (p32), which appears to trigger a T-cell response in PBMC from schizophrenic patients (see Table 2). Another fragment, p1, elicited a smaller response (see Table 2). The responses triggered by the other 36 fragments of HSP60 were similar in magnitude to those obtained with the PBMC from healthy subjects. The normal level of immune response to HSP fragments may be an essential arm in the intricate network of immune homeostasis (Kaufmann 1994). Therefore, an autoimmune response to a distinct HSP60 peptide could originate either from some antigenic cross-reactivity between this peptide and another target molecule, or from an aberration in the delicate balance of the natural immune response to HSP60. On the whole, the present findings provide some support for the autoimmune hypothesis of schizophrenia (Knight 1984, Noy et al. 1994) and suggest the possible existence of a specific target epitope, such as peptide p32.

The putative autoimmune response emanating from HSP60 is at least partially masked by the innate response to HSP60 as a bacterial antigen. This is indicated by the marked overlap between schizophrenic patients and normal subjects in the overt cellular (Fig. 1) and humoral (Table 3) responses to HSP60. In the cellular responses to p32 this overlap was reduced considerably which enabled the assignment of an arbitrary upper value for normal SI (see Fig. 2). However, out of the 32 tested patients only 17 exhibited SI values above this upper normal value. Therefore, at this stage the cellular response to HSP60 or p32 can not provide a reliable tool for biochemical diagnosis of schizophrenia.

It is interesting that the schizophrenic patients manifested increased INF- γ secretion upon HSP60 stimulation compared to the controls (see Fig. 4). INF- γ can affect the neuronal system in several ways. In addition to stimulating differentiation, this cytokine can induce neuronal expression of the nitric oxide synthase (NOS) gene (Minc-Golomb 1996). Cultured neuronal and glial cells were found to respond to IFN- γ by induction of NOS release and subsequent cell death (Dawson et al. 1994, Goodwin et al. 1995, Sato et al. 1995). It was also found that IFN- γ can increase markedly the susceptibility of the human cortical neurons to infection by coronavirus (Collins 1995). Binding of IFN- γ to its high-affinity receptor induces a rapid activation of phospholipase A2 (PLA2) (Ponzoni and Cornaglia-Ferraris 1993), which could account for the observed increase in PLA2 activity in schizophrenia (Noponen et al. 1993, Brunner and Gattaz 1995, Gattaz et al. 1995). In neurons, membrane-bound PLA2 plays an essential role in signal transduction by affecting neurotransmitter release and receptor sensitivity, with an overall reduction in neurotransmission (including dopaminergic), which in turn, may result in dopaminergic alterations with schizophrenic symptoms (Brunner and Gattaz 1995, Gattaz et al. 1995).

We have previously detected abnormal humoral and cellular autoimmune responses against autologous thrombocytes in schizophrenic patients (Deckmann et al. 1996). Of key importance remains the question whether the p32 peptide of HSP60 cross reacts immunologically with a specific thrombocyte epitope, which might initiate the autoimmune response. This possibility is currently under investigation in our laboratory. A number of other questions related to the possible relevance of autoimmunity to the psychopathological features of schizophrenia remain to be investigated. These include the mode of propagation of the autoimmune response from the periphery to the CNS and the identification of the CNS target antigen. Once these issues are clarified and further confirmed by independent routes, it might be possible to consider immunological regimens for the treatment of schizophrenia.

Acknowledgements We thank Dr. Alpha Peled and Mrs. Anna Gelfand for their technical assistance. M. S. is the Olin-Sang Professor of Leukemia Research.

References

- Abrams JS, Roncarolo MG, Yssel H, Andersson U, Gleich GJ, Silver JE (1992) Strategies of anti-cytokine monoclonal antibody development: Immunoassay of IL-10 and IL-5 in clinical samples. *Immunol Rev* 127:5–24
- Allebeck P, Rodvall Y, Wistedt B (1985) Incidence of rheumatoid arthritis among patients with schizophrenia, affective psychosis and neurosis. *Acta Psych Scand* 71:615–619
- Amar A, Zohar M, Tiwari J, Brautbar B (1988) HLA and schizophrenia in Israel. *Isr J Med Sci* 24:28–31
- American Psychiatric Association (1994) *Diagnostic and Statistical Manual of Mental Disorders*, 4th ed. Washington, DC: American Psychiatric Press
- Anderton SM, van der Zee R, Prakken B, Noordzij A, van Eden W (1995) Activation of T cells recognizing self 60-kD heat shock protein can protect against experimental arthritis. *J Exp Med* 181:943–952
- Birk OS, Elias D, Weiss AS, Rosen A, van der Zee R, Walker MD, Cohen IR (1996) NOD mouse diabetes: The ubiquitous mouse hsp60 is a beta-cell antigen of autoimmune T cells. *J Autoimmun* 9:159–166
- Boog CJ, E. R. de Graeff-Meeder ER, Lucassen MA, van der Zee R, Voorhorst-Ogink S, van Kooten PJS, Geuze HJ, van Eden W (1992) Two monoclonal antibodies generated against human HSP60 show reactivity with synovial membranes of patients with juvenile chronic arthritis. *J Exp Med* 175:1805–1810
- Brunner J, Gattaz WF (1995) Intracerebroventricular injection of phospholipase A2 inhibits apomorphine-induced locomotion in rats. *Psychiatry Res* 58:165–169
- Chengappa KN, Ganguli R, Yang ZW, Shurin G, Brar JS, Rabin BS (1995) Impaired mitogen (PHA) responsiveness and increased autoantibodies in Caucasian schizophrenic patients with the HLA B8/DR3 phenotype. *Biol Psychiatry* 37:546–549
- Coffey CE, Sullivan JL, Rice JR (1983) T lymphocytes in schizophrenia. *Biol Psychiatry* 18:113–119
- Cohen IR (1991) Autoimmunity to chaperonins in the pathogenesis of arthritis and diabetes. *Annu Rev Immunol* 9:567–589
- Collins AR (1995) Interferon gamma potentiates human coronavirus OC43 infection of neuronal cells by modulation of HLA class I expression. *Immunol Invest* 24:977–986
- Danieli MG, Candela M, Ricciatti AM, Reginelli R, Danieli G, Cohen IR, Gabrielli A (1992a) Antibodies to mycobacterial 65kDa heat shock protein in systemic sclerosis (scleroderma). *J Autoimmun* 5:443–452
- Danieli MG, Markovitz D, Gabrielli A, Corvetta A, Giorgi PL, Van der Zee R, van Embden JD, Danieli G, Cohen IR (1992b) Juvenile rheumatoid arthritis patients manifest immune reactivity to the mycobacterial 65-kDa heat shock protein, to its 180–188 peptide, and to a partially homologous peptide of the proteoglycan link protein. *Clin Immunol Immunopathol* 64:121–128
- Dawson VL, Brahmabhatt HP, Mong JA, Dawson TM (1994) Expression of inducible nitric oxide synthase causes delayed neurotoxicity in primary mixed neuronal-glial cortical cultures. *Neuropharmacology* 33:1425–1430
- De Graeff-Meeder ER, Van der Zee R, Rijkers GT, Schuurman HJ, Kuis W, Bijlsma JWJ, Zegers BJM, van Eden W (1991) Recognition of human 60 kD heat shock protein by mononuclear cells from patients with juvenile chronic arthritis. *Lancet* 337:1368–1372
- Deckmann M, Shinitzky M, Leykin I, Cheng D, Guy J, Avnon M, Salganik I, Amiri Z, Schlossberg A, Leib E, Rafael C (1996) Humoral and cellular response against autologous platelets in schizophrenia – clinical and pathophysiological implications. *Italian J of Psychiatry and Behavioural Sciences* 6:29–34
- DeLisi LE, King AC, Targum S (1985) Serum immunoglobulin concentrations in patients admitted to an acute psychiatric inpatient service. *Br J Psychiatry* 145:661–665
- DeNagel DC, Pierce SK (1993) Heat shock proteins in immune responses. *Crit Rev Immunol* 13:71–81
- Direskeneli H, Hasan A, Shinnik T, Mizushima Y, van der Zee R, Fortune F, Stanford MR, Lehner T (1996) Recognition of B-Cell Epitopes of the 65 kDa HSP in Behçet's Disease. *Scand J Immunol* 43:464–471
- Dudani AK, Gupta RS (1989) Immunological characterization of a human homolog of the 65-kilodalton mycobacterial antigen. *Infect Immun* 57:2786–2793
- Dvorakova M, Majsky A, Zvolosky P (1989) Incidence of HLA-DR antigens in endogenous psychoses. *Cesk Psychiatr* 85:217–222
- Eaton WW, Hayward C, Ram R (1992) Schizophrenia and rheumatoid arthritis: a review. *Schizophr Res* 6:181–192
- Elias D, Markovits D, Reshef T, Van der Zee R, Cohen IR (1990) Induction and therapy of autoimmune diabetes in the non-obese diabetic (NOD/Lt) mouse by a 65kDa heat shock protein. *Proc Natl Acad Sci USA* 87:1576–1580
- Elias D, Reshef T, Birk OS, van der Zee R, Walker MD, Cohen IR (1991) Vaccination against autoimmune mouse diabetes using a T cell epitope of human 65kDa heat-shock protein. *Proc Natl Acad Sci USA* 88:3088–3091

- Elias D, Markus H, Reshef T, Ablamunits V, Cohen IR (1995) Induction of diabetes in standard mice by immunization with the p277 of a 60-kDa heat shock protein. *Eur J Immunol* 25: 2851–2857
- Finney GOH (1989) Juvenile onset diabetes and schizophrenia? *Lancet* 18: 1214–1215
- Ganguli R, Rabin BS, Kelly RH, Lyte M, Ragu U (1987) Clinical and laboratory evidence of autoimmunity in acute schizophrenia. *Ann NY Acad Sci USA* 496: 676–685
- Ganguli R, Rabin BS (1989) Increased serum interleukin 2 receptor concentration in schizophrenic and brain-damaged subjects. *Arch Gen Psychiatry* 46: 292
- Ganguli R, Rabin BS, Belle SH (1989) Decreased interleukin-2 production in schizophrenic patients. *Biol Psychiatry* 26: 427–430
- Ganguli R, Brar JS, Solomon W, Chengappa KN, Rabin BS (1992) Altered interleukin-2 production in schizophrenia: association between clinical state and autoantibody production. *Psychiatry Res* 44: 113–123
- Ganguli R, Brar JS, Chengappa KR, DeLeo M, Yang ZW, Shurin G, Rabin BS (1995) Mitogen-stimulated interleukin-2 production in never-medicated, first-episode schizophrenic patients. The influence of age at onset and negative symptoms. *Arch Gen Psychiatry* 52: 668–672
- Gattaz WF, Schmitt A, Maras A (1995) Increased platelet phospholipase A2 activity in schizophrenia. *Schizophr Res* 16: 1–6
- Goodwin JL, Uemura E, Cunnick JE (1995) Microglial release of nitric oxide by the synergistic action of beta-amyloid and IFN-gamma. *Brain Res* 692: 207–214
- Hasan A, Fortune F, Wilson A, Warr K, Shinnick T, Mizushima Y, van der Zee R, Stanford MR, Sanderson J, Lehner T (1996) Role of $\gamma\delta$ T cells in pathogenesis and diagnosis of Behçet's disease. *Lancet* 347: 789–794
- Heath RG, McCarron KL, O'Neil CE (1989) Antiseptal brain antibody in IgG of schizophrenic patients. *Biol Psychiatry* 25: 725–733
- Henneberg AE, Horter S, Ruffert S (1994) Increased prevalence of anti-brain antibodies in the sera from schizophrenic patients. *Schizophr Res* 14: 15–22
- Hermann E, Lohse AW, Van der Zee R, van Eden W, Mayet WJ, Probst P, Poralla T, Meyer zum Büschenfelde KH, Fleisher B (1991) Synovial fluid-derived Yersinia-reactive T cells responding to human 65-kDa heat-shock protein and heat-stressed antigen-presenting cells. *Eur J Immunol* 21: 2139–2143
- Heufelder AE, Wenzel BE, Bach RS (1992) Cell surface localization of a 72 kilodalton heat shock protein in retroocular fibroblast from patients with Graves' ophthalmopathy. *J Clin Endocrinol Metab* 74: 732–736
- Hunt DW, Corson L, Baker HD, Levy JG, Petty RE (1993) Relationship between collagen-induced and adjuvant arthritis in the Lewis rat. *J Autoimmun* 6: 691–700
- Jindal S, Dudani AK, Singh B, Harley CB, Gupta RS (1989) Primary structure of a human mitochondrial protein homologous to the bacterial and plant chaperonins and to the 65-kilodalton mycobacterial antigen. *Mol Cell Biol* 9: 2279–2283
- Kaufmann SHE (1990) Heat shock proteins and the immune response. *Immunol Today* 11: 129–136
- Kaufmann SHE (1994) Heat Shock Proteins and Autoimmunity: A Critical Appraisal. *Int Arch Allergy Immunol* 103: 317–322
- Kay SR, Poler LA, Eiszbein A (1990) Positive and negative syndrome scale (PANSS). Toronto Multi-Health System Inc
- Kilidireas K, Latov N, Strauss DH, Gorig AD, Hashim GA, Gorman JM, Sadiq SA (1992) Antibodies to the human 60 kDa heat-shock protein in patients with schizophrenia. *Lancet* 340: 569–572
- Knight JG, Adams DD (1982) The genetic basis of autoimmune disease. *Ciba Foundation Symposium* 90: 35–56
- Knight JG (1984) Is Schizophrenia an Autoimmune Disease? – A Review. *Meth and Find Exp Clin Pharmacol* 6: 394–403
- Knight JG, Knight A, Menkes DB, Mullen PE (1990) Autoantibodies against brain septal region antigen specific to unmedicated schizophrenia? *Biol Psychiatry* 28: 467–474
- Lamb JR, Young DB (1990) T cell recognition of stress proteins: a link between infectious and autoimmune diseases. *Mol Biol Med* 7: 311–321
- Levine J, Susnovski M, Handzel ZT, Leykin I, Shinitzky M (1994) Treatment of schizophrenia with an immunosuppressant. *Lancet* 34: 59–60
- Levine J, Gutman J, Feraro R, Levy P, Kimhi R, Leykin I (1997) Side effect profile of azathioprine in the treatment of chronic schizophrenic patients. *Neuropsychobiology* 36: 172–176
- Life PF, Bassey EOE, Gaston HJS (1991) T-cell recognition of bacterial heat shock proteins in inflammatory arthritis. *Immunol Rev* 121: 113–135
- Maclaren NK, Atkinson MA (1997) Insulin-dependent diabetes mellitus: the hypothesis of molecular mimicry between islet cell antigens and microorganisms. *Mol Med Today* 3: 76–83
- Malck-Ahmadi P (1985) Rheumatoid arthritis and schizophrenia: are they mutually exclusive? *Semin Arthritis Rheum* 15: 70–72
- Masserini C, Vita A, Basile R, Morselli R, Boato P, Peruzzi C, Pugnetti L, Ferrante P, Cazzullo CL (1990) Lymphocyte subsets in schizophrenic disorders. Relationships with clinical, neuromorphological and treatment variables. *Schizophr Res* 3: 269–275
- Mazeh D, Sirota P, Patya M, Novogrotsky A (1998) Antibodies to neuroblastoma cell line proteins in patients with schizophrenia. *J Neuroimmunol* 84: 218–222
- McAllister CG, van Kammen DP, Rehn TJ, Miller AL, Gurklis J, Kelley ME, Yao J, Peters JL (1995) Increases in CSF levels of interleukin-2 in schizophrenia: effects of recurrence of psychosis and medication status. *Am J Psychiatry* 152: 1291–1297
- Mellso GW, Koadlow L, Syme J, Whittingham S (1974) Absence of rheumatoid arthritis in schizophrenia. *Aust NZJ Med* 4: 247–252
- Minc-Golomb D, Yadid G, Tsarfati I, Resau JH, Schwartz JP (1996) In vivo expression of inducible nitric oxide synthase in cerebellar neurons. *J Neurochem* 66: 1504–1509
- Noponen M, Sanfilippo M, Samanich K, Ryer H, Ko G, Augrist B, Wolkin A, Duncan E, Rotrosen J (1993) Elevated PLA2 activity in schizophrenic and other psychiatric patients. *Biol Psychiatry* 34: 641–649
- Noy S, Achiron A, Laor N (1994) Schizophrenia and Autoimmunity – A Possible Etiological Mechanism? *Neuropsychobiology* 30: 157–159
- Nyland H, Naess A, Lunde H (1980) Lymphocyte subpopulations in peripheral blood from schizophrenic patients. *Acta Psychiatr Scand* 61: 313–318
- Oscan ME, Taskin R, Banoglu R, Batacan M, Tuncer E (1996) HLA Antigens in Schizophrenia and Mood Disorders. *Biol Psychiatry* 39: 891–895
- Ostenberg E (1978) Schizophrenia and rheumatic disease. A study on the concurrence of inflammatory joint diseases and a review of 58 case-records. *Acta Psych Scand* 58: 339–359
- Pandey RS, Gupta AK, Chaturvedi UC (1981) Autoimmune model of schizophrenia with special reference to anti-brain antibodies. *Biol Psychiatry* 16: 1123–1136
- Pervin K, Childerstone A, Shinnick T, Mizushima Y, van der Zee R, Hasan A, Vaughan R, Lehner T (1993) T cell epitope expression of mycobacterial and homologous human 65-kilodalton heat shock protein peptides in short term cell lines from patients with Behçet's Disease. *J Immunol* 151: 2273–2282
- Ponzoni M, Cornaglia-Ferraris P (1993) Interferon-gamma-stimulated and GTP-binding proteins-mediated phospholipase A2 activation in human neuroblasts. *Biochem J* 294: 893–898
- Rajagopalan S, Zordan T, Tsokos GC, Datta SK (1990) Pathogenic anti-DNA autoantibody-inducing T helper cell lines from patients with active lupus nephritis: Isolation of CD4⁺8⁻ T helper cell lines that express the $\gamma\delta$ T-cell antigen receptor. *Proc Natl Acad Sci USA* 87: 7020
- Rapaport MH, McAllister CG, Pickar D, Nelson DL, Paul SM (1989) Elevated levels of soluble interleukin 2 receptors in schizophrenia. *Arch Gen Psychiatry* 46: 291–292

- Res PCM, Thole JER, De Vries RRP (1991) Heat shock proteins in immunopathology. *Current Opinion in Immunology* 3:924–929
- Rothermundt M, Arolt V, Weitzsch C, Eckhoff D, Kirchner H (1996) Production of cytokines in acute schizophrenic psychosis. *Biol Psychiatry* 40:1294–1297
- Sato I, Kim Y, Himi T, Murota S (1995) Induction of calcium-independent nitric oxide synthase activity in cultured cerebellar granule neurons. *Neurosci Lett* 184:145–148
- Schwarz MJ, Riedel M, Gruber R, Müller N, Ackenheil M (1998) Autoantibodies against 60-kDa heat shock protein in schizophrenia. *Eur Arch Psychiatry Clin Neurosci* 248:282–288
- Selmaj K, Brosnan CF, Raine CS (1991) Colocalization of Lymphocytes bearing γ/δ T-cell receptor and heat shock protein hsp65⁺ oligodendrocytes in multiple sclerosis. *Proc Natl Acad Sci USA* 88:6452–6456
- Shintani F, Kanba S, Maruo N, Nakaki T, Nibuya M, Suzuki E, Kinoshita N, Yagi G (1991) Serum interleukin-6 level in schizophrenic patients. *Life Sci* 49:661–664
- Sorokina TT, Evsegneev RA, Levin VI, Semenov GV (1987) Features of the distribution of HLA-antigens among patients with endogenous psychoses. *Zh Nevropatol Psychiatr* 87:885–888
- Spector TD, Silman AJ (1987) Does the negative association between rheumatoid arthritis and schizophrenia provide clues to the aetiology of rheumatoid arthritis? *Br J Rheumatol* 26:307–310
- Spector TD, Silman AJ (1990) Rheumatoid arthritis, diabetes, and schizophrenia. *Lancet* 335:228–229
- Srivastava PK (1994) Heat shock proteins in immune response to cancer: the Fourth Paradigm. *Experientia* 50:1054–1060
- Stanford MR, Kasp E, Whiston R, Hasan A, Todryk S, Shinnik T, Mizushima Y, Dumonde DC, van der Zee R, Lehner T (1994) Heat shock protein peptides reactive in patients with Behçet's disease are uveitogenic in Lewis rats. *Clin Exp Immunol* 97:226–231
- Sugerman AA, Southern DL, Curran JF (1982) A study of antibody levels in alcoholic, depressive and schizophrenic patients. *Ann Allergy* 48:166–171
- van Eden W, Thole JE, van der Zee R, Noordzij A, van Embden JD, Hensen EJ, Cohen IR (1988) Cloning of the mycobacterial epitope recognised by T lymphocytes in adjuvan arthritis. *Nature* 331:171–173
- Vartanyan ME, Kolyaskina GI, Lozovsky OV, Burbaeva G S, Ignatov SA (1978) Aspects of humoral and cellular immunity in schizophrenia. *Birth Defects* 14:339–364
- Villemain F, Chatenoud L, Galinowski A, Homo-Delarche F, Ginestet D, Loo H, Zarifian E, Bach JF (1989) Aberrant T cell-mediated immunity in untreated schizophrenic patients: deficient interleukin-2 production. *Am J Psychiatry* 146:609–616
- Vinogradov S, Gottesman II, Moises HW, Nicol S (1991) Negative association between schizophrenia and rheumatoid arthritis. *Schizophr Bull* 17:669–678
- Wilke I, Arolt V, Rothermundt M, Weitzsch C, Hornberg M, Kirchner H (1996) Investigations of cytokine production in whole blood cultures of paranoid and residual schizophrenic patients. *Eur Arch Psychiatry Clin Neurosci* 246:279–284
- Winfield JB (1989) Stress proteins, arthritis, and autoimmunity. *Arthritis Rheum* 32:1497–1504
- Wright P, Murray RM (1993) Schizophrenia: prenatal influenza and autoimmunity. *Ann Med* 25:497–502
- Wright P, Sham PC, Gilvarry CM, Jones PB, Cannon M, Sharma T, Murray RM (1996) Autoimmune diseases in the pedigrees of schizophrenic and control subjects. *Schizophr Res* 20:261–267
- Xu Q, Dietrich H, Steiner HJ, Gown AM, Schoel B, Mikuz G, Kaufmann SHE, Wick G (1992) Induction of arteriosclerosis in normocholesterolemic rabbits by immunization with heat shock protein 65. *Arteriosclerosis Thromb* 12:789–799
- Yang ZW, Chengappa KN, Shurin G, Brar JS, Rabin BS, Gubbi AV, Ganguli R (1994) An association between anti-hippocampal antibody concentration and lymphocyte production of IL-2 in patients with schizophrenia. *Psychol Med* 24:449–455
- Young RA (1990) Stress proteins and immunology. *Annu Rev Immunol* 8:401–420
- Young D, Lathigra R, Hendrix R, Sweetser D, Young RA (1988) Stress proteins are immune targets in leprosy and tuberculosis. *Proc Natl Acad Sci USA* 85:4267–4270