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

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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 ± SE) for schizophrenic patients and 2.23 ± 0.24 (mean ± 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

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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 antibrain autoantibodies in schizophrenic patients (Heath et al. 1989, Knight et al. 1990, Henneberg et al. 1994, Yang et al. 1994), which can induce behavioral

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. 1992 a, 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 temperature 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 ± 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 IgA_1/A_2 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 obtain 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% CO_2 at 37 °C. Each well contained 200,000 PBMC suspended in 200 µl of RPMI 1640 medium supplemented with 2% heat inactivated human AB serum, 2 mM L-glutamine, penicillin (100 units/ml), streptomycin (100 µg/ml), gentamycin sulfate (50 µg/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 µg/ml for HSP60 and 25 µg/ml for the peptide fragments of HSP60. Wells with Tetanus Toxoid (TT) (1 µg/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 µCi per well of ^{3}H-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 overnight 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 biotinilated 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 H_2O_2 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 substracted from the overall reading.

Dot-blot experiments

One microliter of different concentrations of HSP (1 µg/µl, 100 ng/µl, 10 ng/µl, 1 ng/µl, 100 pg/µl, 10 pg/µl, and 1 pg/µl), 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 µl of sera from schizophrenic or control subjects by overnight incubation at room temperature, with gentle shaking in 800 µl 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 $CaCl_2$, pH 8.0, for 4 hours at room temperature with 3 mg/ml 4-chloronaphthol (Sigma Chemical Co., USA) in methanol containing 3% H_2O_2 as the color releasing substrate.

Detection of anti-HSP60 antibodies

Human HSP60 or TT (as a positive control antigen), diluted in PBS (10 µg/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/

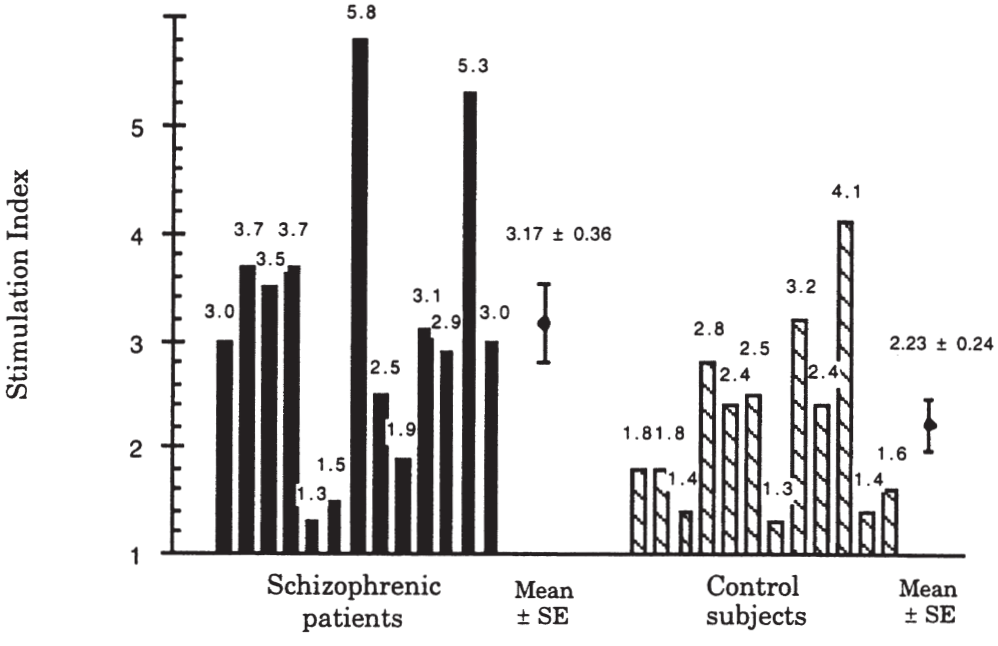

Fig. 1 Stimulation of lymphocytes from schizophrenic patients (n = 13) and control subjects (n = 12) by human HSP60. The difference in the responses was significant (P = 0.0457)

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 H_2O_2 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 ± 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 ± 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 ± 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 ^{3}H-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 μg/ml of different fragments of HSP60. Indices higher than 2 are marked.

No.	Aminoacid sequence	Peptide fragment (residues)	Mean SI
1.	MLRLPTVFRQMRPVSRVLAP	1– 20	2.5
2.	RVLAPHLTRAYAKDVKFGAD	16– 35	1.0
3.	KFGADARALMLQGVDLLADA	31– 50	1.2
4.	LLADAVAVTMGPKGRTVIIE	46– 65	1.3
5.	TVIIEQSWGSPKVTKDGVTV	61– 80	1.3
6.	DGVTVAKSIDLKDKYKNIGA	76– 95	1.3
7.	KNIGAKLVQDVANNTNEEAG	91–110	1.3
8.	NEEAGDGTTTATVLARSIAK	106–125	1.1
9.	RSIAKEGFEKISKGANPVEI	121–140	1.0
10.	NPVEIRRGVMLAVDAVIAEL	136–155	1.2
11.	VIAELKKQSKPVTTPEEIAQ	151–170	1.0
12.	EEIAQVATISANGDKEIGNI	166–185	1.2
13.	EIGNIISDAMKKVGRKGVI	181–199	1.1
14.	RKGVITVKDGKTLNDELEII	195–214	1.3
15.	ELEIIEGMKFDRGYISPYFI	210–229	1.3
16.	SPYFINTSKGQKCEFQDAYV	225–244	1.2
17.	QDAYVLLSEKKISSIQSIVP	240–259	1.5
18.	QSIVPALEIANAHRKPLVIIA	255–275	1.9
19.	LVIIAEDVDGEALSTLVLNR	271–290	1.2
20.	LVLNRLKVGLQVVAVKAPGF	286–305	1.3
21.	KAPGFGDNRKNQLKDMAIAT	301–320	1.1
22.	MAIATGGAVFGEEGLTLNLE	316–335	1.0
23.	TLNLEDVQPHDLGKVGEVIV	331–350	1.0
24.	GEVIVTKDDAMLLKGKGDKA	346–365	1.2
25.	KGDKAQIEKRIQEIIEQLDV	361–380	1.1
26.	EQLDVTTSEYEKEKLNERLA	376–395	1.0
27.	NERLAKLSDGVAVLKVGGTS	391–410	1.8
28.	VGGTSDVEVNEKKDRVTDAL	406–425	1.0
29.	VTDALNATRAAVEEGIVLGG	421–440	1.5
30.	IVLGGGCALLRCIPALDSLT	436–455	1.0
31.	LDSLTPANEDQKIGIEIIKR	451–470	1.0
32.	EIIKRTLKIPAMTIAKNAGV	466–485	3.2
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.	PGMGAMGGMGGGMGGGMF	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

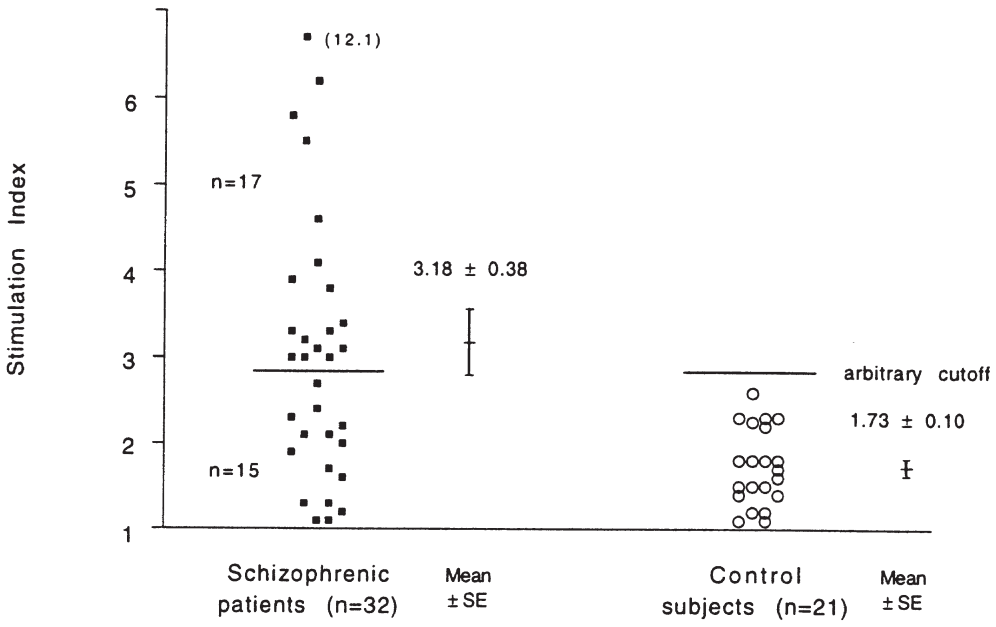

Fig. 2 Stimulation of lymphocytes from schizophrenic patients (n = 32) and control subjects (n = 21) by peptide p32 of HSP60 (see Table 1). The difference in response between the groups was highly significant (P = 0.0006). An arbitrary cutoff for the upper normal value was taken as SI ≈ 3. Among the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

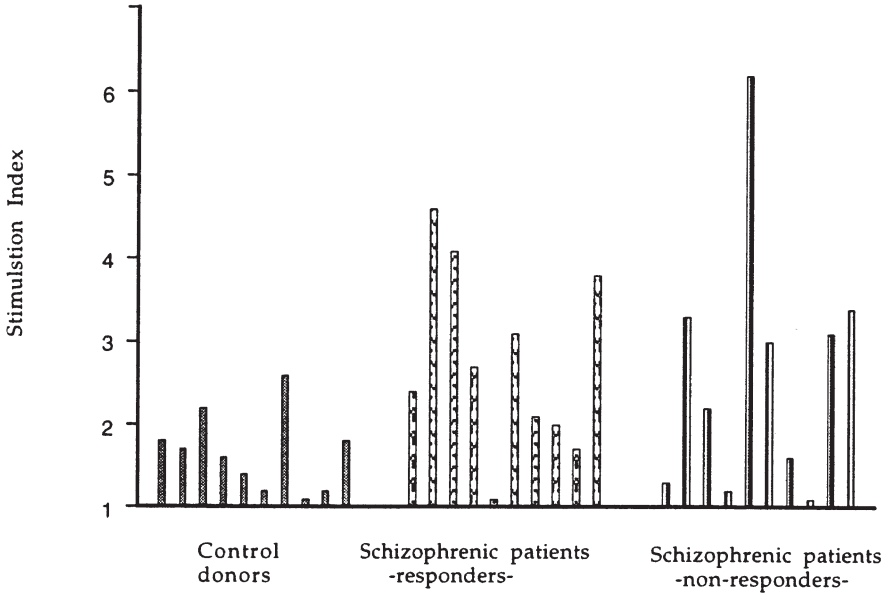

Fig. 3 Stimulation of lymphocytes from responders (n = 10) and non-responders (n = 10) schizophrenic and normal control subjects (n = 10) by peptide p32 of HSP60 (see Table 2). The difference in response between responders and control subjects was significant (P = 0.0147), while the difference between 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 ^{3}H-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).

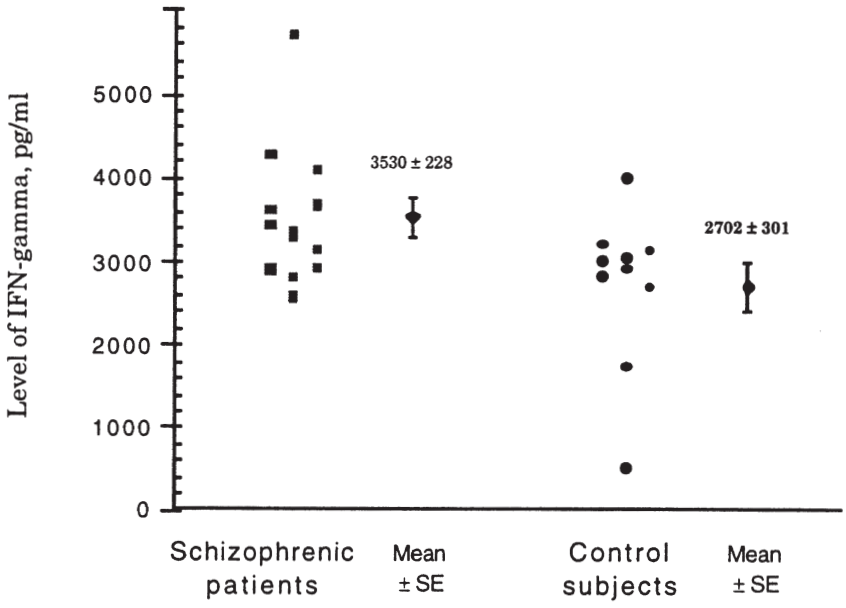

Fig. 4 IFN-γ secretion of lymphocytes from schizophrenic patients and control subjects after stimulation by human HSP60. The difference in values between the groups was significant (P = 0.0358)

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

	IgG	IgM	IgA
Schizophrenic patients	0.043 ± 0.012	0.254 ± 0.030	0.122 ± 0.027
Normal subjects	0.110 ± 0.025 (2 cases with exceptionally high values)	0.373 ± 0.054	0.047 ± 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.

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