



Mother–child immunological interactions in early life affect long-term humoral autoreactivity to heat shock protein 60 at age 18 years

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Abstract

The presence of anti-heat shock protein 60 (Hsp60) antibodies in healthy individuals and the association of these antibodies with diseases such as arthritis and atherosclerosis are well documented. However, there is limited population-level data on interindividual variation in anti-Hsp60 levels. We investigated the influence of early-life factors on IgG reactivity to human Hsp60 at age 18 years. A population-based prospective birth cohort study included 5914 births in the city of Pelotas, Brazil, in 1982. Early-life exposures were documented during home visits in childhood. In 2000, 79% of all males in the cohort were traced. Sera from a systematic 20% sample (411 subjects) were analyzed. Anti-Hsp60 total IgG reactivity was determined by ELISA. Data were analyzed using analysis of variance and generalized linear models. Anti-Hsp60 reactivity was lognormally distributed and showed a significant direct correlation with low birthweight ($p = 0.039$) and total duration of breastfeeding ($p = 0.018$), of which only the latter remained significant after adjustment for potential confounders. Reactivity was not associated with asthma, pneumonia, diarrhea, or early-life malnutrition. Mother–child immunological interactions, rather than infection/disease factors seem to be associated with reactivity to Hsp60 later in life. This is in agreement with the hypothesis that maternal antibodies influence future antibody profile.

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1. Introduction

Heat shock proteins (HSPs) are a family of phylogenetically conserved proteins present in all cells of all living organisms, performing various intracellular and systemic homeostatic functions. They are so named because of their increased expression in stress situations, including heat shock, oxidative stress, ultraviolet radiation, and viral infection [1]. In addition to their role in intracellular maintenance, heat shock proteins

also seem to interact extensively with the immune system, through both the innate and adaptive branches. This is especially the case for the 60 kDa member of this family, heat shock protein 60 (Hsp60), which has been reported to be involved in both triggering and helping downmodulate immune responses [2]. Hsp60 is also part of the ‘immunological homunculus,’ postulated by Cohen and Young [3], which consists of a limited set of self-antigens preferentially recognized by a regulatory network of autoreactive T and B cells.

Naturally occurring autoantibodies to human Hsp60 are found in most, if not all individuals [4], and seem to remain at stable levels for long periods of time [5]. This is compatible with the concept that antibody autoreactivity may contribute to the maintenance of homeostasis [6]. On the other hand,

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increased levels of anti-Hsp60 antibodies have been reported in a wide variety of diseases, from arthritis, diabetes, and atherosclerosis to schizophrenia and noise-induced hearing loss [7–11]. Whether elevated anti-Hsp60 levels are harmful per se, or whether these represent a healthy response to stress (including disease) is yet to be established.

Few studies investigate factors associated with the broad inter-individual variation seen in anti-Hsp60 levels among healthy subjects. Generally speaking, autoantibody repertoires appear to be established very early in life and to remain essentially constant until advanced age [12]. These so-called ‘natural autoantibodies’ (NAAbs) are present in all individuals in the absence of known immunization, and their origin is still a matter of debate [6]. Data from animal models suggests that the transmission of antibodies from mother to offspring via the placenta and breastfeeding may play an important role in shaping these repertoires [13]. Although autoantibodies to Hsp60 have not traditionally been studied by groups investigating NAAbs, the ubiquity and long-term stability of anti-Hsp60 autoreactivity warrant its study in this context.

Epidemiological studies can help understand interindividual variability in autoantibody levels and their role in health and disease. In the present study, we investigate the association between early life events and total IgG reactivity to human Hsp60 at age 18 years. Our *a priori* hypothesis was that maternal transmission of antibodies would affect anti-Hsp60 reactivity in adult life. This is, to our knowledge, the first study of the influence of such early events on humoral autoreactivity at a later age in humans.

2. Materials and methods

A population-based prospective birth cohort study included 5914 births in the city of Pelotas, Brazil, in 1982 [14]. Children were weighed soon after delivery, and gestational age was calculated based on the date of the last menstrual period. Early life exposures were measured during home visits in childhood. In 2000, 79% of all males in the cohort were traced, and serum was obtained from all subjects. A 20% systematic sample was selected by numbering subjects by order of birth and selecting all subjects whose records ended in 2 or 7. This procedure yielded a final sample size of 411 subjects whose sera were analyzed.

Recombinant human Hsp60 was produced in *E. coli* bacteria (gene provided by AC Camargo, Butantan Institute, São Paulo, Brazil) and purified by Ni–histidine-tag affinity chromatography in our laboratory. Protein purity was >90% (SDS-PAGE/densitometry) (Fig. 1A). A single band was present upon Western blotting with an anti-human Hsp60 antibody not crossreactive with Hsp60 from *E. coli* (SPA-806, Stressgen Bioreagents, Canada) (Fig. 1B).

Total IgG reactivity was determined by enzyme-linked immunosorbent assay (ELISA). Plates were coated with 1.5 µg/ml recombinant human Hsp60 and blocked with 0.25% gelatin (Sigma–Aldrich, USA) and serum samples were added in duplicate at 1/100 dilution. Antibodies were detected using peroxidase-conjugated rabbit anti-human total IgG (BD

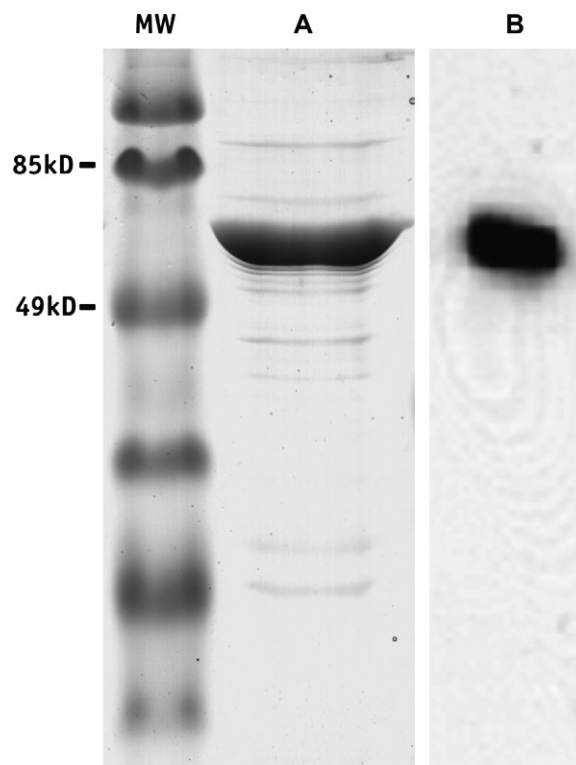


Fig. 1. Purity of recombinant human Hsp60 preparation used for ELISA. (A) SDS-PAGE followed by Coomassie blue staining. Purity was >90%. (B) Western blotting with anti-human Hsp60. MW: molecular weight standard.

Pharmingen, USA) and H₂O₂/o-phenylene-diamine (Sigma–Aldrich, USA) as a substrate. Absorbance (abs.) was read at 492 nm, and the mean abs. of wells without sample (blank) was subtracted from the reactivity of samples in the same plate. Results are expressed as abs. corrected for inter-plate variation using the abs. of a positive control serum (abs. of sample/abs. of control from same plate × mean abs. of controls from all plates). We considered as ‘detectable’ any abs. ≥ 2 times the blank abs. in the same plate.

Several early-life exposures were investigated. Birthweight was measured in 1982 using calibrated pediatric scales. Monthly family income (sum of the wages of all family members) and maternal education (years of schooling) were also recorded during the hospital interview. Total breastfeeding duration in months was collected during the 1983, 1984 and 1986 follow-up visits; the earliest available information on full weaning was used in order to minimize recall bias. Information on environmental variables was collected in 1984: crowding (persons per bedroom), source of water (piped inside the home or other), type of sanitation (flush toilet or other), and housing materials (bricks, wood or improvised materials). In the 1984 visit, parents were asked about whether the child had ever been hospitalized due to diarrhea or to pneumonia; history of asthma or bronchitis episodes was also collected based on parental report. Nutritional status was assessed at the age of four years; height was measured with locally made stadiometers; height for age was assessed using the NCHS reference [15] and stunting was defined as a value below –2 z-scores below the median.

In the year 2000 interview, information was collected on daily smoking, self-reported wheezing in the past year, and skin color (white, black, mixed, or other); the latter was also self-referred, but showed a high level of agreement with skin color assessed by the interviewer ($\kappa = 0.83$). Anthropometric examinations included *standing height* measured using a CMS (London, UK) stadiometer, and weight measured with an electronic Tanita Body Fat Analyzer scale (model TBF-305; Tokyo, Japan). All subjects were measured by the same examiner. The subject's body mass index (BMI) was determined by dividing weight (kg) by the square of height (m). Subjects with BMI between 25 and 29.9 were considered as overweight; those with BMI ≥ 30 were considered as obese.

Data were analyzed using Pearson correlation coefficients, analysis of variance, and generalized linear models, to allow adjustment for socioeconomic variables. All of the above described variables were considered as potential confounders of the association between the exposure variables (birthweight and breastfeeding) and anti-Hsp60 reactivity; those associated (with a $p < 0.20$) both with exposure and outcome were included as confounding variables in the multivariable analyses [16]. Variables that reflect exposures or conditions arising after birthweight and breastfeeding duration had been established (such as childhood illnesses and adult characteristics, e.g. smoking and body mass index) were not considered as confounders but as potential mediating factors in the associations between reactivity to Hsp60 and either birthweight or breastfeeding [17].

The study was approved by the Federal University of Pelotas Medical Research Committee and written informed consent was obtained.

3. Results

Anti-Hsp60 reactivity was log-normally distributed (geometric mean 0.544; range 0.034, 2.597) (Fig. 2A), and was detectable in all but two subjects. There was a monotonic increase in mean reactivity according to categories of birthweight ($p = 0.15$) and duration of breastfeeding ($p = 0.035$) (Table 1; Fig. 2B). There were also significant direct correlations between reactivity and both birthweight in grams (g) ($r = 0.103$; $p = 0.039$) and total duration of breastfeeding in months ($r = 0.119$; $p = 0.018$) (Table. 1).

Table 2 shows that the association between family income at birth and anti-Hsp60 reactivity had a p -value of 0.07, although there was no clear dose-response trend. There was no association between reactivity and either maternal schooling, skin color, or any other socioeconomic or environmental variables (Tables 2 and 3).

We sought associations between reactivity to Hsp60 and several indicators of disease collected in early life, including history of asthma, pneumonia and diarrhea hospital admissions, and presence of childhood malnutrition. None of these associations approached statistical significance (Table 4). Also, no associations were found with current overweight, obesity, daily smoking, or wheezing in the previous year (data not shown).

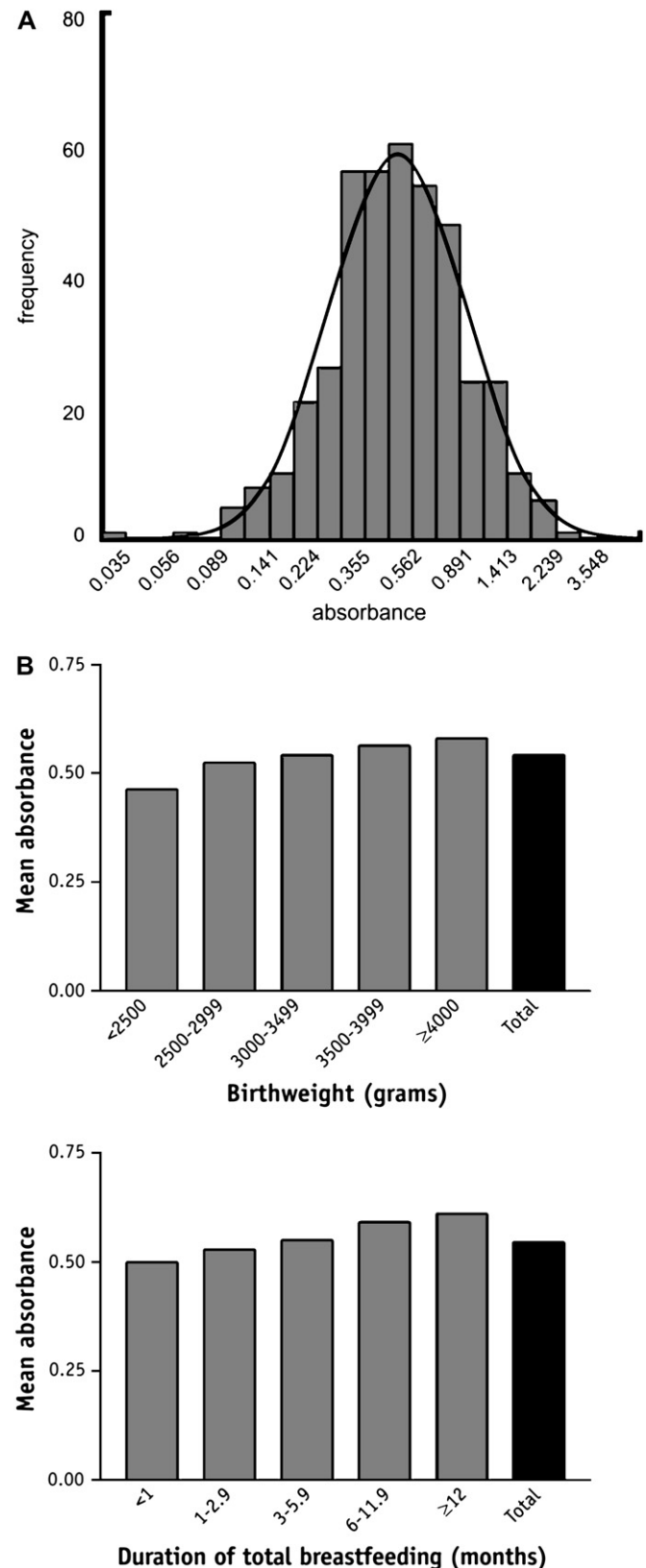


Fig. 2. (A) Frequency distribution of cases according to IgG reactivity to human Hsp60. The solid line represents a lognormal distribution. (B) Mean IgG reactivity to human Hsp60 according to categories of birthweight (in grams) and duration of total breastfeeding (in months). The leftmost bar in each group represents mean reactivity in the entire sample.

Table 1
Mean (95% CI) IgG reactivity to human Hsp60 according to birthweight and duration of total breastfeeding. Pelotas, Brazil, 2000

	Mean absorbance	95% CI		<i>N</i>
<i>Birthweight (grams)^a</i>				
<2500	0.464	0.352	0.611	24
2500–2999	0.525	0.463	0.595	86
3000–3499	0.542	0.493	0.596	159
3500–3999	0.564	0.492	0.645	105
≥4000	0.581	0.458	0.737	32
Total				406
<i>Total breastfeeding duration (months)^b</i>				
<1	0.500	0.443	0.565	100
1–2.9	0.529	0.469	0.596	106
3–5.9	0.551	0.480	0.632	88
6–11.9	0.591	0.493	0.709	46
≥12	0.610	0.515	0.722	54
Total				394

^a ANOVA for linear trend $p = 0.152$; Pearson correlation coefficient with continuous variable = 0.103; $p = 0.039$.

^b ANOVA for linear trend $p = 0.035$; Pearson correlation coefficient with continuous variable = 0.119; $p = 0.018$.

Family income at birth and type of housing in early life were both associated at the $p < 0.20$ level with reactivity to Hsp60 and with birthweight, thus fulfilling the criteria for potential confounding. The association between birthweight and anti-Hsp60 reactivity was still significant after adjustment for income ($p = 0.048$) but not after additional adjustment for housing ($p = 0.175$). On the other hand, the association between duration of breastfeeding in months and reactivity to Hsp60 remained statistically significant after adjustment for family income ($p = 0.031$). This association persisted ($p = 0.048$) even when all seven early life variables (Tables 2 and 3) as well as birthweight were adjusted for.

4. Discussion

The finding that anti-Hsp60 was log-normally distributed in a population sample is in agreement with previous studies [4]. We considered total IgG reactivity to human Hsp60 as a continuous variable, which is appropriate to understanding variability within populations [18]. This is in contrast with the predominant approach used in immunology regarding the analysis of antibodies to microbial antigens, in which subjects are usually dichotomized into reactive or non-reactive on the basis of cut-off points defined by the mean and variability of healthy individuals. Some of the earlier studies on anti-Hsp60 have used the latter approach, thus reporting very low prevalence of positive reactions [10].

The extensive homology between human and bacterial Hsp60 has led to the hypothesis that anti-self Hsp60 reactivity may result largely from repeated stimulation with crossreactive bacterial homologues, in a phenomenon called *antigen mimicry* [19]. Indeed, antibodies that react with both human and bacterial Hsp60 have been reported in different settings [20,21], but whether these antibodies were elicited by bacterial or human antigens remains unclear. In our sample, levels of anti-Hsp60 humoral reactivity were not associated with indicators

Table 2
Mean (95% CI) IgG reactivity to human Hsp60 according to socioeconomic variables. Pelotas, Brazil, 2000

	Mean absorbance	95% CI		<i>N</i>
<i>Family income at birth (quintiles)</i>				<i>p</i> ^a = 0.07
1	0.465	0.394	0.549	66
2	0.630	0.552	0.719	92
3	0.534	0.474	0.602	82
4	0.526	0.463	0.599	85
5	0.542	0.466	0.631	81
Total				406
<i>Maternal schooling (years)</i>				<i>p</i> ^a = 0.32
0–4	0.540	0.479	0.609	123
5–8	0.535	0.492	0.581	182
9–11	0.482	0.372	0.624	40
≥12	0.611	0.524	0.712	60
Total				405
<i>Skin color (self-reported)</i>				<i>p</i> ^a = 0.41
White	0.533	0.496	0.572	287
Black	0.607	0.507	0.727	67
Mixed	0.499	0.396	0.629	34
Other	0.548	0.432	0.694	18
Total				406

^a ANOVA for heterogeneity.

of childhood infections nor with closely related variables such as malnutrition or exposure to poor environmental conditions, suggesting a minor, if any, role for bacterial cross-activation in the early determination of anti-Hsp60 levels, at least in this population. We were unable to evaluate BCG vaccination – which could also affect anti-Hsp60 reactivity by antigen mimicry – since subjects in this cohort were only vaccinated at about age 7, therefore, after the 1986 follow-up visit. However,

Table 3
Mean (95% CI) IgG reactivity to human Hsp60 according to environmental variables. Pelotas, Brazil, 2000

	Mean absorbance	95% CI		<i>N</i>
<i>Crowding (persons/bedroom)</i>				<i>p</i> ^a = 0.28
<2	0.518	0.452	0.592	75
3–3.9	0.545	0.500	0.593	225
≥4	0.579	0.502	0.669	72
Total				372
<i>Water source</i>				<i>p</i> ^b = 0.76
Piped inside the home	0.548	0.512	0.587	319
Other	0.532	0.444	0.639	54
Total				373
<i>Type of sanitation</i>				<i>p</i> ^b = 0.59
Flush toilet	0.542	0.506	0.581	320
Other	0.570	0.477	0.681	53
Total				373
<i>Type of housing</i>				<i>p</i> ^b = 0.18
Bricks	0.526	0.487	0.568	256
Wood	0.581	0.505	0.668	78
Improvised	0.628	0.510	0.772	37
Total				371

^a ANOVA for linear trend.

^b ANOVA for heterogeneity.

Table 4
Mean (95% CI) IgG reactivity to human Hsp60 according to nutritional status and morbidity variables. Pelotas, Brazil, 2000

	Mean absorbance	95% CI	N
<i>History of asthma or bronchitis in first 20 months of life</i> $p^a = 0.46$			
No	0.535	0.498 0.574	296
Yes	0.567	0.488 0.659	80
Total			376
<i>Hospital admission due to diarrhea in first 20 months of life</i> $p^a = 0.98$			
No	0.541	0.506 0.579	354
Yes	0.544	0.437 0.677	22
Total			376
<i>Hospital admission due to pneumonia in first 20 months of life</i> $p^a = 0.72$			
No	0.544	0.507 0.584	314
Yes	0.527	0.452 0.615	62
Total			376
<i>Presence of stunting at age 4 years</i> $p^a = 0.52$			
Normal	0.538	0.503 0.575	344
Stunted	0.580	0.462 0.729	32
Total			376

^a ANOVA for heterogeneity.

coverage in Brazil at this time was high, and is unlikely to be associated with birthweight or breastfeeding.

Another potential source of anti Hsp60 humoral reactivity is inflammation, which leads to greater expression and availability of HSPs for interaction with the immune system [22]. This hypothesis is in agreement with the reported correlation between levels of anti-Hsp60 antibodies and of circulating soluble Hsp60 [23]. In our cohort, however, anti-Hsp60 humoral reactivity was not associated with inflammatory conditions either during childhood (asthma, infections) or adult life (wheezing and smoking). Obesity in adult life, another postulated inflammatory condition [24], also failed to show an association with the outcome. Whether or not our subjects, aged 18 years, are old enough for such associations to be detected is unclear.

Instead, our data suggest that mother–child immunological interactions may be more closely related to humoral reactivity to Hsp60 later in life than are past or current morbidity. Intensity of reactivity appears to be associated with the duration of breast milk intake. This is in accordance with the hypothesis that the transfer of maternal antibodies early in development plays a role in defining future antibody profile [13,25]. The association with birthweight, another variable that may reflect mother–child interactions, was significant in the crude analysis and after adjustment for family income, but not when other potential confounders were included in the analyses.

The lack of an effect of current measures of morbidity or inflammation supports the idea that antibody repertoires are defined during an early critical period and remain stable thereafter [13]. If confirmed by other studies, the finding that breastfeeding has a measurable association with an immunological parameter as late as age 18 years is remarkable, given the time lapse between exposure and outcome.

As the role and effects of anti-Hsp60 humoral reactivity are not yet fully understood, the implications of our findings are

unclear. Although several studies report associations between elevated anti-Hsp60 humoral reactivity and autoimmune diseases or atherosclerosis [7–9], it is not entirely clear whether these antibodies are a cause or a consequence of the disease process. Moreover, a recent study has reported lower levels of anti-Hsp60 antibodies in subjects with Sjögren's syndrome [26], which suggests that the association between anti-Hsp60 and autoimmune disease may not be as clear-cut.

On the other hand, physiological humoral autoimmunity, i.e. the presence of naturally autoreactive antibodies in healthy individuals, is well documented. Human and animal studies provide evidence for a broad range of reactivities to autoantigens in the absence of autoimmune disease, and propose physiological roles for such antibodies [6,27], suggesting that higher levels of autoantibodies may not necessarily be harmful. Moreover, if Hsp60 is indeed part of a select group of 'regulatory self-antigens' [3], physiological antibody response to this antigen is expected. The idea that humoral reactivity to Hsp60 may be physiological is supported by the fact that all but two of a population-based sample of over 400 18-year old males showed detectable reactivity to Hsp60 at 1/100 serum dilution.

Methodological strengths of our study include its population basis, its prospective design, and the fact that almost 80% of all subjects were traced at the age of 18 years. Follow-up rates did not vary markedly according to social or biological conditions in early life [28]. Limitations of our study include the fact that it was restricted to males. Further studies are required to replicate our findings, and to extend these findings to females. If the association is confirmed, it will be necessary to investigate whether the modulation of humoral reactivity to Hsp60 is an isolated phenomenon, or if similar influences can be found for other self or nonself antigens. Researchers should also attempt to identify which substances present in breast milk may be associated with anti-Hsp60 reactivity.

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References

- [1] Pockley AG. Heat shock proteins as regulators of the immune response. *Lancet* 2003;362:469–76.
- [2] van Eden W, Koets A, van Kooten P, Prakken B, van der Zee R. Immunopotentiating heat shock proteins: negotiators between innate danger and control of autoimmunity. *Vaccine* 2003;21:897–901.
- [3] Cohen IR, Young DB. Autoimmunity, microbial immunity and the immunological homunculus. *Immunol Today* 1991;12:105–10.
- [4] Pockley AG, Bulmer J, Hanks BM, Wright BH. Identification of human heat shock protein 60 (Hsp60) and anti-Hsp60 antibodies in the peripheral circulation of normal individuals. *Cell Stress Chaperones* 1999;4: 29–35.
- [5] Fust G, Beck Z, Banhegyi D, Kocsis J, Biro A, Prohaszka Z. Antibodies against heat shock proteins and cholesterol in HIV infection. *Mol Immunol* 2005;42:79–85.

- [6] Lacroix-Desmazes S, Kaveri SV, Mouthon L, Ayoub A, Malanchere E, Coutinho A, et al. Self-reactive antibodies (natural autoantibodies) in healthy individuals. *J Immunol Methods* 1993;216(1–2):117–37.
- [7] de Graeff-Meeder ER, Rijkers GT, Voorhorst-Ogink MM, Kuis W, van der Zee R, van Eden W, et al. Antibodies to human HSP60 in patients with juvenile chronic arthritis, diabetes mellitus, and cystic fibrosis. *Pediatr Res* 1993;34:424–8.
- [8] Zhu J, Quyyumi AA, Rott D, Csako G, Wu H, Halcox J, et al. Antibodies to human heat-shock protein 60 are associated with the presence and severity of coronary artery disease: evidence for an autoimmune component of atherogenesis. *Circulation* 2003;103:1071–5.
- [9] Ozawa Y, Kasuga A, Nomaguchi H, Maruyama T, Kasatani T, Shimada A, et al. Detection of autoantibodies to the pancreatic islet heat shock protein 60 in insulin-dependent diabetes mellitus. *J Autoimmun* 1996;9:517–24.
- [10] Kilidireas K, Latov N, Strauss DH, Gorig AD, Hashim GA, Gorman JM, et al. Antibodies to the human 60 kDa heat-shock protein in patients with schizophrenia. *Lancet* 1992;340:569–72.
- [11] Yang M, Zheng J, Yang Q, Yao H, Chen Y, Tan H, et al. Frequency-specific association of antibodies against heat shock proteins 60 and 70 with noise-induced hearing loss in Chinese workers. *Cell Stress Chaperones* 2004;9:207–13.
- [12] Mouthon L, Lacroix-Desmazes S, Nobrega A, Barreau C, Coutinho A, Kazatchkine MD. The self-reactive antibody repertoire of normal human serum IgM is acquired in early childhood and remains conserved throughout life. *Scand J Immunol* 1996;44:243–51.
- [13] Lemke H, Lange H. Is there a maternally induced immunological imprinting phase à la Konrad Lorenz? *Scand J Immunol* 1999;50:348–54.
- [14] Victora CG, Barros FC, Lima RC, Horta BL, Wells J. Anthropometry and body composition of 18-year-old males according to breastfeeding duration: a birth cohort study from Brazil. *Br Med J* 2003;327:901–4.
- [15] WHO Expert Committee. Physical status: the use and interpretation of anthropometry: WHO; 1995.
- [16] Maldonado G, Greenland S. Simulation study of confounder-selection strategies. *Am J Epidemiol* 1993;138:923–36.
- [17] Victora CG, Huttly SR, Fuchs SC, Olinto MTA. The role of conceptual frameworks in epidemiological analysis: a hierarchical approach. *Int J Epidemiol* 1997;26:224–7.
- [18] Rose G. Sick individuals and sick populations. *Int J Epidemiol* 2001;30:427–32.
- [19] Handley HH, Yu J, Yu DT, Singh B, Gupta RS, Vaughan JH. Autoantibodies to human heat shock protein (hsp)60 may be induced by *Escherichia coli* groEL. *Clin Exp Immunol* 1996;103:429–35.
- [20] Perschinka H, Mayr M, Millonig G, Mayerl C, van der Zee R, Morrison SG, et al. Cross-reactive B-cell epitopes of microbial and human heat shock protein 60/65 in atherosclerosis. *Arterioscler Thromb Vasc Biol* 2003;23:1060–5.
- [21] Uray K, Hudecz F, Fust G, Prohaszka Z. Comparative analysis of linear antibody epitopes on human and mycobacterial 60-kDa heat shock proteins using samples of healthy blood donors. *Int Immunol* 2003;15:1229–36.
- [22] van Eden W, van der Zee R, Prakken B. Heat-shock proteins induce T-cell regulation of chronic inflammation. *Nat Rev Immunol* 2005;5:318–30.
- [23] Xiao Q, Mandal K, Schett G, Mayr M, Wick G, Oberhollenzer F, et al. Association of serum-soluble heat shock protein 60 with carotid atherosclerosis: clinical significance determined in a follow-up study. *Stroke* 2005;36:2571–6.
- [24] Das UN. Is obesity an inflammatory condition? *Nutrition* 2001;17:953–66.
- [25] Lemke H, Coutinho A, Lange H. Lamarckian inheritance by somatically acquired maternal IgG phenotypes. *Trends Immunol* 2004;25:180–6.
- [26] Shovman O, Sherer Y, Gilbourd B, Gerli R, Bocci EB, delle Monache F, et al. Low levels of heat shock proteins-60 and -65 autoantibodies in Sjgren's syndrome. *Isr Med Assoc J* 2005;7:778–80.
- [27] Quintana FJ, Cohen IR. The natural autoantibody repertoire and autoimmune disease. *Biomed Pharmacother* 2004;58:276–81.
- [28] Victora CG, Barros FC, Lima RC, Behague DP, Gonçalves H, Horta BL, et al. The Pelotas birth cohort study, Rio Grande do Sul, Brazil, 1982–2001. *Cadernos de Saude Publica* 2003;19:1241–56.