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Serum IgG Subclass Levels in a Group of Healthy Thai Children

Jarungchit Ngamphaiboon[1], Apiradee Theamboonlert[2] and Yong Poovorawan[2]

SUMMARY **Gammaglobulins are the major components of the humoral immune response to foreign antigens. Yet, they may cause disease, for example, in certain malignancies or autoimmune disorders. The discovery of IgG subclasses, IgG1-IgG4, has further led to the realization that various gammaglobulin deficiencies may be ascribed to IgG subclass abnormalities. In order to establish a set of reference values in Thai children we have determined the range of total IgG and IgG subclass levels among a cohort of 195 healthy Thai children chosen semi-randomly from those at the Well Child Clinic, Chulalongkorn Hospital, who fitted certain inclusion criteria such as absence of recent infection or history of recurrent infections. The sera obtained were subjected to a laboratory test performed by means of a commercially available kit which uses the radial immunodiffusion technique for distinguishing the different IgG subclasses. The results obtained showed the total immunoglobulin increasing with age, as well as subclasses IgG_1, IgG_2 and IgG_4, whereas subclass IgG_3 remained at an almost constant level, irrespective of the age group tested.**

Gammaglobulins are produced by B lymphocytes and represent the major proteins involved in humoral immunity. Gammaglobulins protect against infections or foreign antigen, but may cause disease in malignancies, autoimmune and other disorders. Terry and Fahey[1] and Gray and Kunkel[2] showed that there are four subclasses of IgG, IgG_1-IgG_4.

IgG subclasses differ in their amino acid sequences. Fairly unique differences exist both in the hinge region and at the C terminus.[3] These differences, as well as the tryptic peptide differences, have been used for typing myeloma proteins to the different subclasses. Other amino acid differences reflect inherited genetic markers (the "Gm" markers). These markers are unique amino acid sequences that are identified by an inhibition of the agglutination reaction. Certain Gm markers are recognized in IgG subclasses 1, 2 and 3, but none in IgG4.

Serum IgG subclass abnormalities have been noted particularly in patients with monoclonal gammopathies and infections (immunodeficiencies), as well as in some other disorders. Of particular interest has been the observation that IgG subclass deficiencies are one of the causes of recurrent infections.[4] In many of these studies, low serum concentrations or absent IgG_2 have been observed in patients with recurrent upper respiratory and pulmonary infections, particularly those due to polysaccharide encapsulated bacteria (*S. pneumoniae*, *H. influenzae*).[5,6] Patients with selective IgG_2 deficiency have been noted to suffer from pulmonary function impairments that progress with age.[7] Various patterns of immunoglobulin defi-

From the [1]Allergy and Immunology Unit, [2]Viral Hepatitis Research Unit, Department of Pediatrics, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand.
Correspondence: Yong Poovorawan

ciencies have been described consisting of normal IgG levels but exhibiting low levels of one or more IgG subclasses (usually IgG_2, IgG_3 and/or IgG_4);[8] low IgG levels and low levels of many IgG subclasses (especially IgG_1,[4] low levels and/or absence of IgG_2, IgG_4 and occasionally IgE and IgD, as well).[7,9]

In order to facilitate the recognition of IgG subclasses in children, we have determined the range of total IgG and IgG subclass protein levels in healthy Thai children of different ages in order to apply them as reference values in Thai children.

MATERIALS AND METHODS

Population study

Before the onset of the study described below, the protocol had been approved by the Ethical Committee, Faculty of Medicine, Chulalongkorn University and Hospital. Informed consent had been obtained from the parents of the respective children involved in the trial.

One hundred and ninety-five healthy children aged between newly delivered and 15 years were selected on a semi-random basis from the Well Child Clinic at Chulalongkorn Hospital, as well as from students attending the government school. The inclusion criteria were: no previous infection within 4 weeks prior to the study, no history of recurrent infections, no history of any systemic illness, and normal results of the physical examination which included normal weight and height with respect to the age group tested.

Clotted blood samples were centrifuged at 1,500 rpm and the sera thus obtained were kept at -70°C until further tested.

Laboratory test

The test was performed by radial immunodiffusion technique, applying the commercially available human IgG and IgG subclasses single dilution kit (BIND A RID™), The Binding Site, Birmingham, England, according to the manufacturer's specifications. All specimens were tested by the same technician.

Statistical analysis

For tabulation, the arithmetic mean of IgG and IgG subclasses, respectively, was calculated per age group. The geometric mean values among the different age groups are depicted in the figures.

RESULTS

Immunoglobulin and IgG subclasses obtained from 195 healthy children aged below 15 years were analyzed, divided between age groups, as shown in Table 1. The main IgG subclass encountered consisted of IgG_1 comprising two thirds of the total IgG, followed by IgG_2, whereas the IgG_3 levels did not show any correlation as to the respective age groups. Total immunoglobulin levels, as well as IgG_1 and IgG_2 subclass levels increased with age among the children tested until reaching a plateau level at the age of approximately 3 to 4 years. The mean IgG_4 levels increase with age, reaching the adult plateau at the age of about 4-6 years.

The scattergram and geometric mean results with standard deviation of total IgG and IgG subclass determined for each age group are shown in Figs. 1 and 2.

DISCUSSION

In the present study, we established the average normal levels of IgG and IgG subclasses in Thai children ranging from newborns to 15-year-old adolescents. In this group of children, 62.9% of IgG belongs to subclass IgG_1, 26.9% to IgG_2, 7.4% to IgG_3 and 2.8% to IgG_4. The results thus obtained are meant to serve as a reference in diagnosing IgG subclass deficiency in children with unexplained recurrent pyogenic infection. The results indicate the normal levels of total IgG, as well as all IgG subclasses except IgG_4, in Thai children to be higher than those measured in Caucasian children,[10] with the mean serum IgG_2 level higher than those obtained for the remaining subclasses. The reason is poorly understood, but it might depend on race, Gm or Km allotypes of immunoglobulin, or environmental factors such as infections which might be different from country to country. The elevated mean serum IgG_2 level could be due to a lower incidence of infections by *Hemophilus influenzae* type B in Thailand,[11] as has also been reported from Hong Kong,[12,13] thus, the IgG_2 subclass level might be affected rather by genetic than environmental factors. The IgG_4 subclass level decreased gradually until reaching the lowest level at the age of 7 to 12 months, as reported from Japan,[14] and increased again gradually with age, reaching a plateau at 4-6 years. Serum levels of the IgG_4 subclass below approximately 0.04 mg/dl were found in 22

Table 1. The mean and standard deviation of serum total immunoglobulin and immunoglobulin subclasses among different age groups in healthy Thai children. The range intervals are in brackets

Age (years)	No. of subjects	Total IgG	IgG_1	IgG_2	IgG_3	IgG_4
0 - 0.5	25	7.02 ± 2.84 (2.75 - 12.43)	4.65 ± 2.25 (2.2 - 8.82)	1.26 ± 0.61 (0.68 - 2.38)	0.92 ± 0.51 (0.01 - 1.94)	0.17 ± 0.19 (0.019 - 0.81)
0.6 - 1	20	9.32 ± 2.99 (6.04 - 16.61)	6.23 ± 2.12 (3.54 - 12.3)	2.05 ± 1.16 (4.24 - 0.77)	0.81 ± 0.44 (0.39 - 1.17)	0.16 ± 0.16 (0.03 - 0.65)
1.1 - 2	25	10.72 ± 2.92 (5.53 - 15.68)	6.77 ± 2.09 (3.15 - 10.3)	2.78 ± 1.05 (0.68 - 5.73)	0.94 ± 0.52 (0.10 - 1.94)	0.22 ± 0.21 (0.02 - 0.81)
2.1 - 3	25	12.49 ± 3.75 (6.40 - 17.82)	8.18 ± 2.69 (4.21 - 11.9)	3.12 ± 1.27 (0.86 - 6.82)	0.82 ± 0.37 (0.15 - 1.59)	0.37 ± 0.29 (0.01 - 0.81)
3.1 - 4	23	10.45 ± 3.46 (5.98 - 21.89)	6.35 ± 2.38 (3.57 - 15.1)	2.85 ± 1.20 (0.68 - 5.21)	0.84 ± 0.42 (0.20 - 1.34)	0.42 ± 0.26 (0.07 - 0.81)
4.1 - 6	24	12.28 ± 3.98 (7.32 - 16.61)	7.78 ± 2.81 (4.44 - 11.9)	3.28 ± 1.16 (1.58 - 4.71)	0.77 ± 0.49 (0.05 - 1.94)	0.49 ± 0.28 (0.01-0.81)
6.1 - 10	28	12.67 ± 3.56 (5.98 - 20.46)	7.87 ± 2.89 (3.78 - 15.8)	3.92 ± 1.57 (1.06 - 8.0)	0.63 ± 0.39 (0.10 - 1.94)	0.30 ± 0.26 (0.01 - 0.81)
10.1 - 15	25	13.93 ± 4.17 (6.86 - 24.75)	8.7 ± 2.76 (3.99 - 17.4)	4.63 ± 1.01 (1.06 - 6.09)	0.81 ± 0.50 (0.20 - 1.9)	0.37 ± 0.27 (0.01 - 0.81)

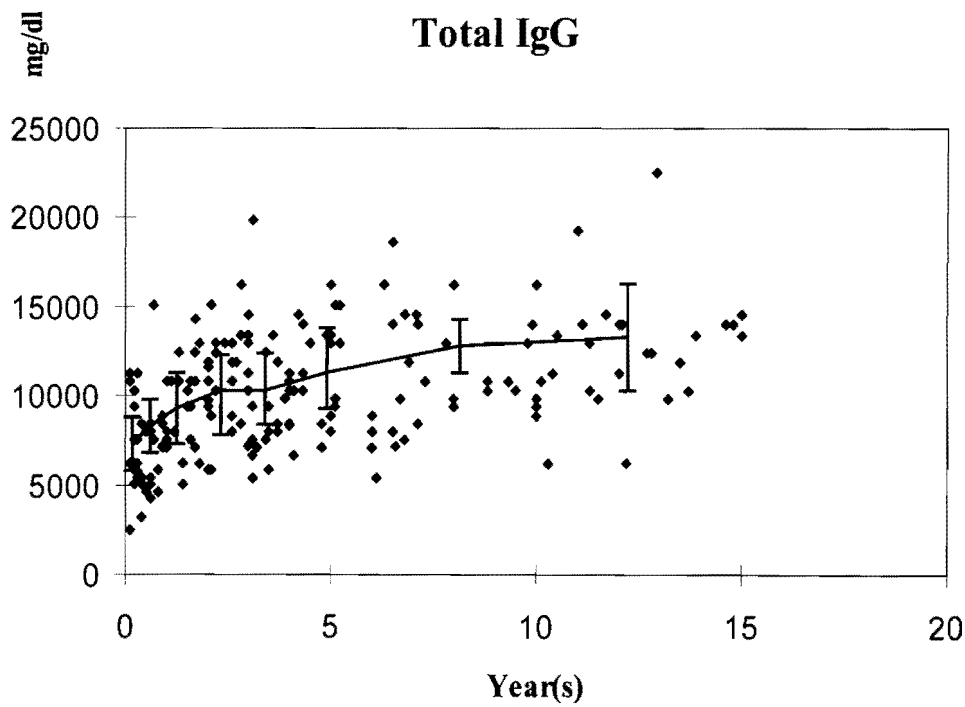
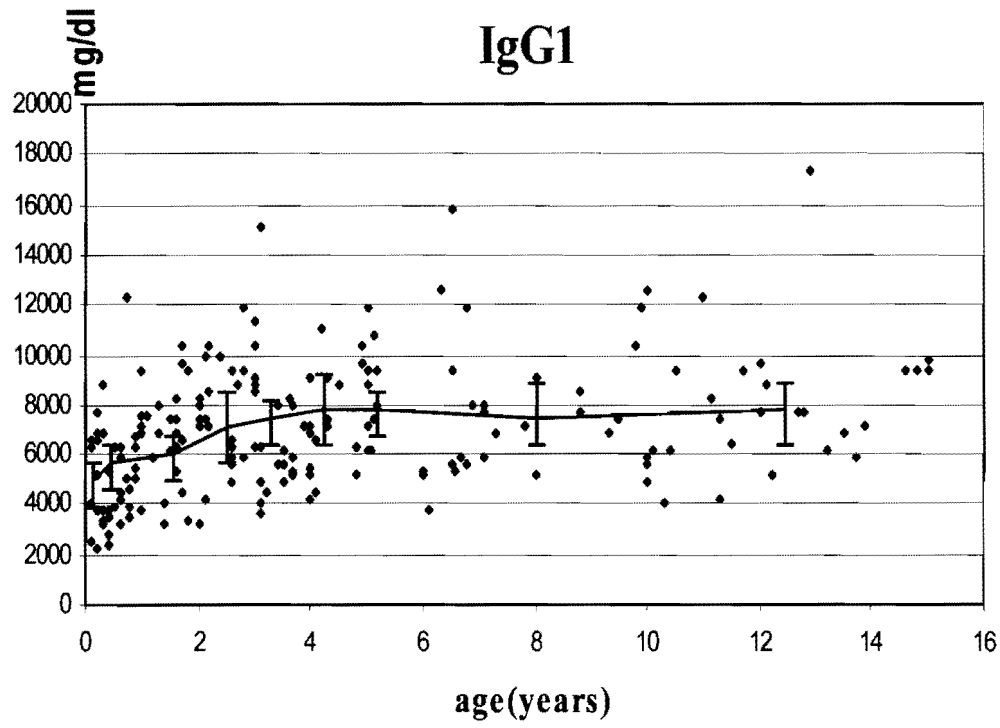
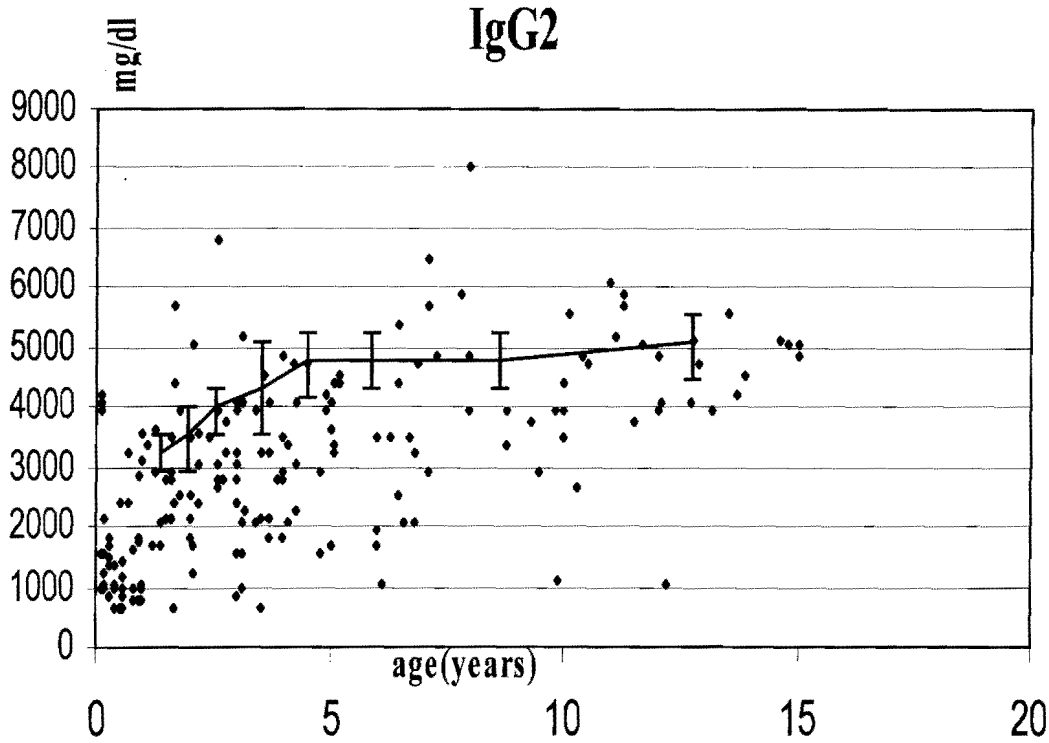


Fig. 1. The geometric mean with standard deviation of total IgG among different age groups in healthy children.

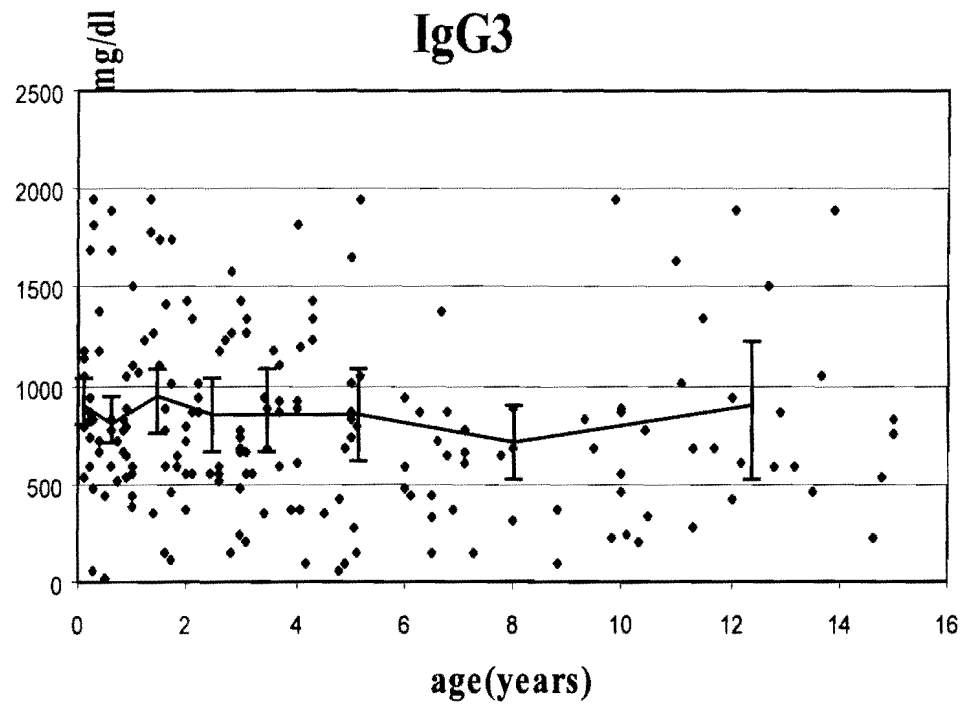


2a

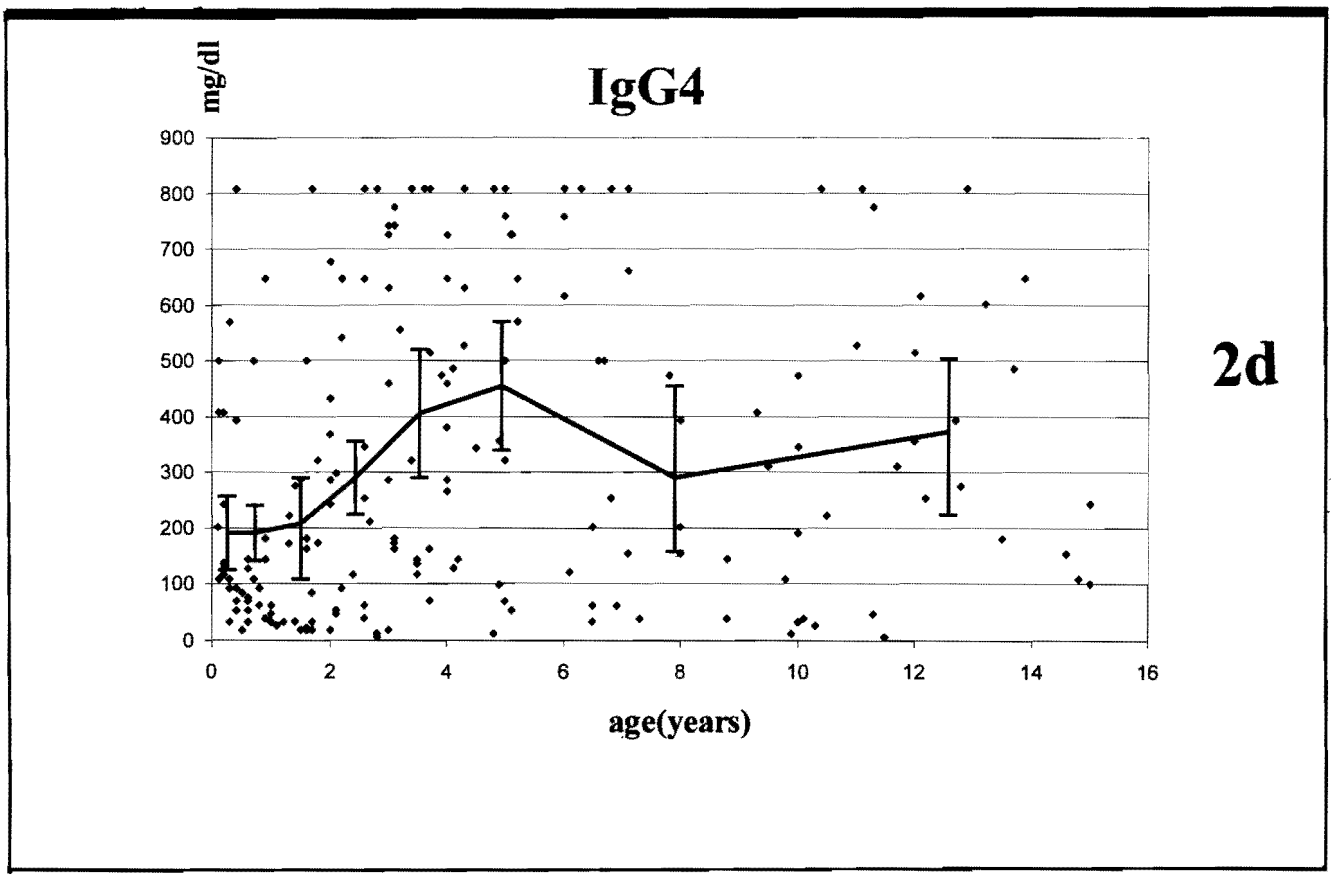
Fig. 2. The geometric mean with standard deviation of IgG subclasses among the different age groups in healthy children (Fig. 2a-2d).


IgG2
mg/dl
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
age(years)
0
5
10
15
20

2b


IgG3
mg/dl
2500
2000
1500
1000
500
0
0
2
4
6
8
10
12
14
16
age(years)

2c



sera in this study (11.28 %). This was similar to the results obtained in the study by Shackelford[15] in that 10% of normal children exhibited the lowest IgG_4 subclass. Compared to the previous study[16] of normal IgG and IgG subclass levels in Thai children between the age of 6 to13 years, we found in this study the mean serum IgG subclass level higher in the IgG_1, IgG_2 subclass, but lower in the total IgG and the IgG_4 subclass. The mean serum IgG_3 level was similar. These findings could not be explained but when we combined the mean levels of IgG_1-IgG_4 of the study mentioned above, they were not correlated to the total IgG level.

Immunoglobulin G is transferred from the mother during the last trimester with its level gradually declining until the age of 4 to 6 months. Hence, the levels of IgG measured within the 0-0.5-year group are necessarily heterogeneous and exhibit considerable variation between the time of birth and the first IgG synthesis by the newborn. Comparing the levels of all IgG subclasses we found the IgG_3 level not increasing with age from the moment of birth, but rather remaining steady until the children had reached adolescence (approximately 15 years), indicating that IgG_3 might well represent the first IgG subclass produced in children.

Recurrent upper and lower respiratory tract infections and chronic rhinosinusitis are very common among Thai children, which might be a consequence of any IgG subclass deficiency (IgG_1-IgG_4).[17,18,19,20,21] There has already been one report of IgG subclass deficiencies associated with bronchiectasis.[22]

We have established the average normal levels of total IgG and IgG subclasses in 195 Thai children aged from birth to 15 years so that IgG subclass deficiencies will be increasingly recognized, especially in association with recurrent infections. This in turn will lead to effective treatment of the respective patients once the diagnosis is confirmed.

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