

Maternal Smoking Leads to Increased Cord Serum IgG3

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IgG subclass levels in cord serum of unselected European newborns were studied in relation to parental smoking. Cord IgG3 was significantly ($P < 0.01$) higher in newborns of smoking mothers ($n = 18$; geom.mean = 87.0%) compared to those of non-smoking mothers ($n = 66$; geom.mean = 61.0%). Maternal smoking did not influence ($P > 0.10$) cord IgG2 or cord IgG4. Paternal smoking did not affect any of the three immunoglobulins in cord serum ($P > 0.10$). These data suggest that tobacco smoking affects IgG3 synthesis in some direct or indirect way.

Key words: cord serum; IgG subclasses; immunoassay; latex agglutination; smoking.

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CLINICAL ASPECTS

It seems that both active and passive smoking affect the humoral and cellular immune system either directly by noxious substances in tobacco smoke or indirectly, e.g. by causing a defective mucosal barrier. These effects are complex and may be both stimulatory and suppressive depending on the dose-time relation of exposure and target organ. Consequently, the smoking status might be important when interpreting IgG subclass data.

Maternal smoking during pregnancy affects the foetus in several ways, some of which are controversial (1, 15) while others are well recognized, e.g. reduced birth weight and head circumference (15). Recently, it has been suggested that maternal smoking also influences the foetal immune system since increased levels of IgA, IgM, IgD and IgE were registered in cord serum of newborns to smoking mothers (7, 19). Also, IgG in cord serum was increased (7), but since IgG passes the placental barrier (30) this could mainly reflect the maternal immune system even though the foetus might also contribute (25). Confusingly, however, active smokers are known to have reduced levels of IgG (2, 9, 11, 20). One possible explanation for these differences is that the IgG subclasses were affected in a different manner by smoking.

Apart from one small study on cord IgG1 (29), and a recent one on all four IgG subclasses

of healthy subjects showing that only IgG1 was affected (21), no data are available on the effects of smoking on the IgG subclasses.

These considerations led us to investigate the effects of parental smoking on the cord serum levels of the IgG subclasses in a randomized sample of European newborns (19).

SUBJECTS AND METHODS

Parental smoking histories were taken from allergy questionnaires in a randomized series of European newborns as previously described (19). No attention was paid to the intensity of smoking and the parents were only considered as smokers or non-smokers following their answer to the question "Are you a smoker?".

The levels of IgG subclasses in cord serum were determined by an automated latex agglutination-inhibition particle counting im-

munoassay (PACIA) essentially as described elsewhere (17). IgG1, IgG2 and IgG3 in cord serum were assayed with polyclonal antisera (Dutch Red Cross and Blood Transfusion Service, Amsterdam) exactly as described (17), whereas for cord IgG4 a monoclonal antibody (BAM 11, Seward Laboratories, London) was used (18). Unfortunately, the linearity of the IgG1 assay was found too unreliable and the results could not be used. The IgG2-assay may be influenced by high levels of IgG4 bearing the nG4m(b)-allotype since the here used sheep anti-IgG2 antiserum (17), like most rabbit anti-IgG2 antisera (10), cross-react with this allotype which is isotypic on IgG2 (14). However, in most cases such IgG4 only contributes to a minor extent to the IgG2 value and should therefore not significantly influence the overall IgG2 results (14). IgG4 was assayed in all available cord sera ($n = 182$), whereas IgG2 and IgG3 were assayed on a randomly taken sample ($n = 84$). The IgG4 results were expressed in $\mu\text{g/ml}$ by reference to a commercial standard (HOO-02, Dutch Red Cross and Transfusion Services, Amsterdam; 0.46 mg/ml) and, IgG2 and IgG3 in percentage of a homemade normal human serum pool. Weight, head circumference and length at birth were taken from medical records whenever available

($n = 175$). Gestational age was available in 161 cases.

The non-parametric Mann-Whitney U-test with corrections for ties was used to evaluate the results, and 2-tailed P -values ≤ 0.05 were considered significant.

RESULTS

Offspring to smoking mothers had reduced weight ($P < 0.025$), head circumference ($P < 0.025$), but not length ($P > 0.05$) at birth as compared with offspring to non-smoking mothers, i.e. 3242 g, 34.2 cm and 50.6 cm ($n = 40$) versus 3454 g, 34.7 cm and 51.2 cm ($n = 135$), respectively. There was no difference ($P > 0.10$) in gestational age between newborns to smoking ($n = 34$; mean = 39.3 weeks) and non-smoking mothers ($n = 127$; mean = 39.3 weeks).

The effects of parental smoking on the levels of IgG2, IgG3 and IgG4 in cord serum are illustrated in Table 1. There was a significantly ($P < 0.01$) higher concentration of cord IgG3 for smoking ($n = 18$; geom. mean = 87.0%) than for non-smoking ($n = 66$; geom. mean = 61.0%) mothers (Fig. 1). However, maternal smoking did not ($P > 0.10$) influence cord IgG2 nor cord IgG4 (Table 1). Paternal smok-

Table 1
Influence of parental smoking on the IgG2, IgG3 and IgG4 levels in cord serum

Immunoglobulin (conc.)	Smoking		<i>N</i>	Geom. mean	+ 2 SD	Mann-Whitney <i>P</i>
	Maternal	Paternal				
Cord IgG2 (%)	+		18	42.6	161.3	NS*
	-		66	44.4	118.0	
		+	32	49.4	152.2	NS
		-	52	41.0	110.2	
Cord-IgG3 (%)	+		18	87.0	209.2	< 0.01
	-		66	61.0	170.9	
		+	32	69.2	216.8	NS
		-	52	63.2	167.2	
Cord IgG4 ($\mu\text{g/ml}$)	+		40	284	4196	NS
	-		142	276	1719	
		+	68	304	2327	NS
		-	114	268	2048	

* NS = Not significant ($P > 0.10$).

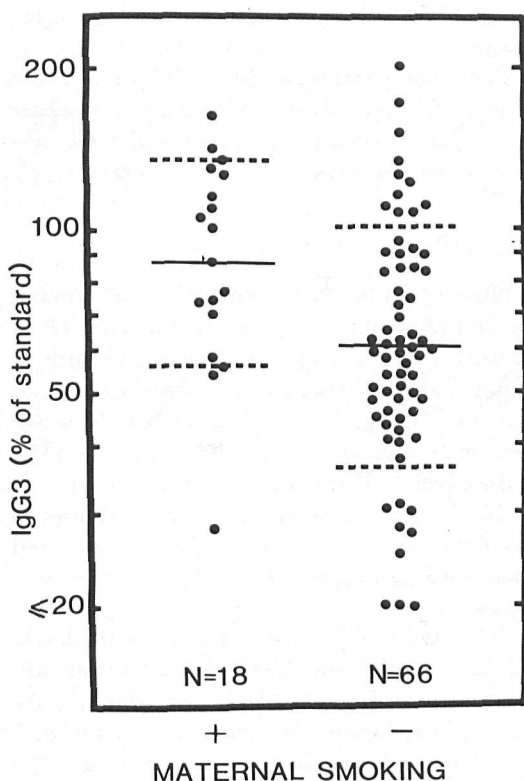


Fig. 1. Influence of maternal smoking on the concentration of IgG3 in cord serum. The horizontal bars indicate the geometrical means $\pm$ 1SD.

ing did not influence any of the three immunoglobulins ($P > 0.10$).

Since maternal allergy might influence cord IgG4 levels, the effect of smoking was compared also among allergic and non-allergic mothers. However, although smoking non-allergic mothers had lower cord IgG4 levels ($n = 30$; geom. mean = 239 $\mu\text{g/ml}$) than non-smoking non-allergic mothers ($n = 103$; geom. mean = 282 $\mu\text{g/ml}$), this difference was not significant ($P > 0.10$).

DISCUSSION

Active smoking probably affects both the humoral and cellular immune system, although these effects are complex and might be both stimulatory and suppressive, depending on the

target organ and the dose-time relation of exposure (12). It is generally accepted that smokers have reduced serum levels of IgG, IgM and IgA (2, 9, 11, 20) but increased levels of IgD (4, 20) and IgE (3, 40). Recently, however, contradictory results on IgG, IgM and IgA have appeared showing that these three immunoglobulins were raised in the sera of smoking mothers (7) as well as in the corresponding cord sera (7). Likewise, maternal smoking seems also to stimulate foetal synthesis of IgE and IgD (19).

Very little data are available on what effect tobacco smoking has on the IgG subclass levels. In a very recent study on all four subclasses of healthy subjects, only serum IgG1 was affected in smokers, and found significantly increased (21). In one small study, only cord IgG1 was assayed but found non-affected (29). Unfortunately, we were unable to reliably assay IgG1 in the present study, but we found that maternal smoking significantly increased the cord serum level of IgG3 leaving IgG2 and IgG4 unaffected, whereas paternal smoking did not influence any of the three immunoglobulins. Our results of cord serum are in agreement with those of Merrill et al. in adults (21) except that they did not find any influence on IgG3. However, their mean values of serum IgG3 were unexpectedly low (30–50 $\mu\text{g/ml}$), perhaps indicating some assay problems. Although, our anti-IgG3 antiserum might contain unwanted anti-isoallotypic antibodies which could give increased IgG3 via increased IgG1, this seems unlikely since this antiserum did not react with any out of several sera containing 50 IgG1, 13 IgG2 and 5 IgG4 myeloma proteins, respectively (17). Problems related to anti-allotypic antibodies against IgG3 cannot totally be excluded though.

It is generally considered that most IgG of the four subclasses in cord serum is of maternal origin (30). However, a small contribution from the foetus is quite possible (25). Thus, it seems likely that our results in cord serum mainly reflect direct or indirect effects of smoking on the maternal immune system.

If the stimulatory effect of smoking on IgG1 and IgG3 is true, and since IgG1 is the

predominant subclass, it is somewhat confusing that most previous studies claim reduced serum IgG of active smokers (2, 9, 11, 20). However, recently, increased serum IgG levels were reported in paired cord/maternal sera of smoking mothers (7). The explanations for these discrepancies might be several and interrelated. Pregnant women (8) and their fetuses (7) may for unknown reasons, perhaps hormonal (8, 39), respond differently to smoke exposure. Alternatively, there might be a dose-response effect as previously observed for IgE and IgD (3, 4). Indeed, IgD levels were three times higher in pregnant women at the end of the third trimester as compared to controls (13), and there was a trend towards lower IgG levels in both cord serum (7, 29) and maternal serum (7) with increased intensity of maternal smoking. Another possible explanation for the discrepancies may lie in individual differences among the IgG subclasses to be affected by smoking, and, subsequently, between the techniques and antisera used to measure IgG in sera with different subclass ratios. For instance, it has been shown that anti-IgG antisera do not proportionally recognize IgG myeloma proteins of different subclasses (22).

Although the reason for our finding of elevated cord IgG3 might be non-immunological, at least two perhaps synergistically acting immunological causes seem plausible. Substances in tobacco smoke may directly disturb at the regulatory level, probably T-cells (12, 29, 33), and recent data suggest that IgG1 and IgG3 synthesis is more T-cell dependent than IgG2 and IgG4 synthesis (32). Furthermore, IgG1 and IgG3 responses are not seldom associated (23, 35, 36), probably indicating similar regulatory mechanisms of these two immunoglobulins. Elevated IgG3 may also be secondary and caused by the increased frequency of viral and bacterial infections observed among smokers (27). Such infections might trigger both IgG1 (16, 23, 37) and IgG3 (6, 24, 37) antibody production, and increased IgG3 levels have been registered in patients with various recurrent and chronic infections (34). A role for IgG3 in lung host defense is further suggested by the relatively high incidence of

IgG3-deficiency in various infection-prone individuals (28). Moreover, Merrill et al. presented evidence of a local production of IgG3 in the lung (21), and receptors on alveolar macrophages have been demonstrated to have a particular affinity for IgG3-complexes (26). These considerations advocate a protective role for IgG3 in the lung.

On the other hand, IgG3 may be of pathophysiological importance and involved in tissue damage. It is a strong complement-fixing antibody, which via immune complexes can induce severe type III inflammation by producing anaphylatoxins and recruited inflammatory cells. IgG3 is increased in various diseases involving tissue damage, e.g. farmer's lung disease (35), allergic bronchopulmonary aspergillosis (36), and pigeon breeder's disease (31). IgG3 has also been found as the main subclass in deposits in the kidneys of patients with glomerulonephritis (5).

Finally, it is interesting to note that the incidence of allergic alveolitis is higher among non-smokers (20, 38), which implies that smoking in some way affects the pathogenesis of these diseases. Whether this effect bears any relation to increased IgG3 among smokers, is quite possible, and needs further study. In any event, when interpreting IgG1 and IgG3 subclass data, it would be prudent to consider the smoking status of the subjects.

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