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Significance of the differences in the prevalence of anti-HLA antibodies in matched pairs of mother's and cord blood

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ABSTRACT

The presence of IgG against pathogens in the cord blood (CB) of vaccinated mothers is attributed to transplacental transfer. However, previous studies using lymphocytotoxicity assay showed anti-HLA IgG in mother's blood (MB) but not in CB, perhaps due to non-transfer of anti-HLA IgG or assay limitations in detecting anti-HLA IgG. Anti-HLA IgG of native and purified sera of 16 MB and CB pairs were measured using an array of microbeads coated with HLA-I/-II molecules on a Luminex platform. Two cases showed no anti-HLA-I IgG in either MB or CB; four MB cases displayed polyallelic HLA-reactive IgG, with negligible or no reactivity by the corresponding CB sera. Notably, anti-HLA-I reactivity in cases 3–6/11/12 and anti-HLA-II reactivity in cases 1/3/4/6/8/11–13 were restricted to CB, with lower or no HLA-reactivity in MB. Mothers' HLA typing is done for HLA-A*, HLA-B* and DRB1* alleles. The mother in case 14 carried DRB1*11:01, the allele-reactive IgG is seen in both native and the purified fraction of sera of MB but not in CB. Also in cases 15 (DRB1*01:01) and 16 (B*49:01 and DBR1*07:01), the allele-reactive IgGs are seen in both native and purified fractions of MB but not in CB confirming the earlier reports on the absence of materno-fetal transfer of anti-HLA IgG. However, the mother of case 6 is homozygous for DRB1*03:01 and the allele-reactive IgG occurred in both MB and CB, confirming the presence of anti-HLA autoantibodies. In Case 13, the mother (HLA-A*24 and HLA-A*52) and CB carried allele-reactive IgG in both native and purified sera, indicating the possible occurrence of transplacental transfer of the IgG. Further confirmation is restricted by the paucity of detailed molecular HLA typing for both the parents and fetuses. While 37.5% of the native IgG in CB and 18.8% in MB showed DRB3*03:01 reactivity, 100% of purified IgG from both CB and MB showed anti-DRB3*03:01 and anti-DPA1*02:01\DPB1*23:01 antibodies. Several CB cases showed high-prevalence IgG reacting to a single allele of HLA-I and/or HLA-II with minimal or no cross-reactive IgG in CB or in the MB, suggesting the presence of *de novo* antibodies, possibly against non-inherited maternal HLA or inherited parental HLA haplotypes by the fetus.

1. Introduction

Antibodies (IgG) against diphtheria, pertussis toxin, influenza B, and *Neisseria meningitidis* were found in matched pairs of mother's blood (MB) and cord blood (CB) of 197 Dutch women who received vaccinations before age 14 [1] and also against measles, rubella, varicella-zoster, mumps and polio in matched pairs of MB and CB [2]. The presence of all these antibodies in CB is attributed to the "transplacental transfer" of IgG from mother to fetus.

However, when the matched pairs of MB and CB were examined for anti-HLA IgG by lymphocytotoxicity assay, the anti-HLA IgG were found in MB but not CB, suggesting that anti-HLA antibodies are not transferred from mother to fetus [3–12]. Analysis of HLA antibodies in eight matched pairs of MB and CB – by lymphocytotoxicity testing on a panel of 88-125HLA-serotyped donors – showed seven cases of anti-HLA IgG in MB but not CB. However in one case, a single anti-HLA antibody (A11) was identified in both CB and MB, which was attributed to an antigen foreign to the fetus or to antibodies present in the mother, possibly due to previous pregnancy [6]. And, indeed, a fetus inherits paternal HLA where each can be different from the maternal HLA. Subsequent lymphocytotoxicity studies concluded that the presence of HLA antibodies in MB was consequent to pregnancy [7–9]. To our knowledge, no report documents the transfer of anti-HLA IgG from mother to fetus or the production of anti-HLA IgG by the fetus, although fetal spleen cells

Abbreviations: IgG, Immunoglobulin (G); HLA, Human Leukocyte antigen; β2m, β2-microglobulin; MB, Mother's blood; CB, cord blood; SHB, single HLA-coated bead.

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Table 1
Cases of matched pairs of MB and CB and their TFL and hospital ID with HLA typing.

TFL-ID		Milan Hospital ID			A	A	B	B	DRB1	DRB1
# 1	# 019	Pair 19	MICB 019	MICB-0120080620						
	# 261	Pair 19	MICB 261	MICB-0120080620	2	26	15	56	0801	1103
# 2	# 047	Pair 47	MICB 047	MICB-0120080752						
	# 257	Pair 47	MICB 257	MICB-0120080752	3	11	8	44	0301	0405
# 3	# 006	Data not available								
	# 258									
# 4	# 025	Pair 25	MICB 025	MICB-0120080711						
	# 266	Pair 25	MICB 266	MICB-0120080711	11	30	13	35	1104	
#5	# 037	Pair 37	MICB 037	MICB-0120080725						
	# 264	Pair 37	MICB 264	MICB-0120080725	23	69	7	15	1124	1501
# 6	# 004	Pair 4	MICB 004	MICB-0120080642						
	# 251	Pair 4	MICB 251	MICB-0120080642	1	2	8		**0301**[a]	
# 7	# 002	Pair 2	MICB 002	MICB-0120080641						
	# 265	Pair 2	MICB 265	MICB-0120080641	2	33	1402	56	0301	1104
# 8	# 024	Pair 24	MICB 024	MICB-0120080675						
	# 262	Pair 24	MICB 262	MICB-0120080675	24	31	51	56	0401	1501
# 9	# 036	Pair 36	MICB 036	MICB-0120080170						
	# 254	Pair 36	MICB 254	MICB-0120080170	2		7	51	1101	1501
# 10	# 012	Pair 12	MICB 012	MICB-0120080621						
	# 252	Pair 12	MICB 252	MICB-0120080621	2	31	51		1101	1104
# 11	# 010	Pair 10	MICB 010	MICB-0120080662						
	# 260	Pair 10	MICB 260	MICB-0120080662	1	3	7	1517	1301	1302
# 12	# 009	Pair 9	MICB 009	MICB-0120080678						
	# 259	Pair 9	MICB 259	MICB-0120080678	2	30	13	15	0701	1104
# 13	# 035	Pair 35	MICB 035	MICB-0120080692						
	# 253	Pair 35	MICB 253	MICB-0120080692	1	**24**[b]	**52**[b]	37	0701	15
# 14	# 027	Pair 27	MICB 027	MICB-0120080169						
	# 263	Pair 27	MICB 263	MICB-0120080169	**1**[b]	30	40		0401	**1101**[b]
# 15	# 30	Pair 30	MICB 030	MICB-0120080738						
	# 255	Pair 30	MICB 255	MICB-0120080738	31	**68**[b]	15	56	**0101**[b]	0405
# 16	# 049	Pair 49	MICB 049	MICB-0120080745						
	# 256	Pair 49	MICB 256	MICB-0120080745	2		38	**4901**[b]	**0701**[b]	1301

[a] IgG antibody against the allele is seen in CB.
[b] IgG antibody against the allele is seen in MB.

[13] and endothelial cells [14] are capable of synthesizing IgG as early as week 20 of gestation.

In the last decade, detecting HLA antibodies has been highly improved by the sensitive Luminex® multiplex single HLA-coated bead (SHB) assay, each bead coated with one of the many HLA-A/-B/-Cw/-DRB1/-DRB3,4,5/-DQ/-DP molecules, as dimers or monomers and HLA-I with or without β2-microglobulin (β2m) [15,16]. The assay significantly clarified the profiles of anti-HLA-I and -II IgG in therapeutic Intravenous Immunoglobulins [17,18], and if applied to the sera of matched pairs of MB and CB, it may provide a decisive tool for understanding transplacental transfer and the possible fetal generation of anti-HLA IgG.

Another impetus for this investigation was our previous findings. While examining non-alloimmunized adult male sera and their corresponding purified IgG eluates with the SHB assay, a remarkable diversity of naturally occurring anti-HLA-I and II antibodies reacting both to "one's own HLA" and allo-HLA was observed [17,18]. Often, the reactivity of IgG purified from sera was higher than that of the native sera due to masking of HLA-reactive IgG in the native sera by serum peptides, such as those derived from target antigens or antibodies. In addition, a 100% incidence of IgG against DRB3*03:01 and DPA1*02:01\DPB1*23:01 was observed in the purified fractions of IgG of all males. In fact, even the IgG purified from the pooled sera of thousands of donors and supplied as intravenous immunoglobulin (IVIg) showed 100% incidence of these two HLA-II antibodies [18]. Possibly, in MB and CB, IgG might be reacting to DRB3*03:01 and DPA1*02:01\DPB1*23:01, either as transplacental transfer or *de novo* IgGs.

Table 2
Categorization of cases based on incidence of HLA-I and HLA-II reactive IgG in the native and purifed IgG from the sera of mother's (MB) and cord blood (CB).

Groups	Cases	HLA-Ia ($n = 97$)	HLA-II ($n = 91$)
1	1, 2	0	<3
2	3, 4, 5	1	2–6
3	6, 7, 8	4	2–15
4	9, 10, 11, 12, 13	10–36	2–4
5	14, 15, 16	20–57	18–51

Comparing the profiles of both native and purified anti-HLA-I/-II IgG in the sera of 16 matched pairs of MB and CB, two distinct patterns of anti-HLA IgG were noted in the native and purified sera of the matched pairs, in addition to IgG reacting to DRB3*03:01 and DPA1*02:01\ DPB1*23:01 in MB and CB.

2. Methods

2.1. Source of mother's and cord blood sera

Sera from 16 pairs of CB and MB, both obtained after delivery with informed consent, were provided by Professor Francesca