LEUCOCYTIC ISO-IMMUNIZATION IN PREGNANCY

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SUMMARY

The results of a study on leucocytic iso-immunization in pregnancy and on the antibody characteristics of the sera produced are reported.

The investigation was carried out on 774 puerperae, making use of the cytotoxicity reaction. The results may be summed up as follows: *a*) pregnancy constitutes an important cause of leucocytic iso-immunization; *b*) leucocytic iso-immunization may occur either in primiparae or multiparae, but the relation between the two iso-immunization percentages was not statistically significant; *c*) the lymphocytotoxis sera may show direct antibody specificity towards either the known antigens as listed in the Major Histocompatibility System, or else antigens not yet defined; *d*) the spectrum of antibody specificity varies in relation to the temperature at which the reaction occurs but does not seem to be influenced significantly by the number of pregnancies; *e*) the antibody specificities obtained, do not always correspond to those expected on the basis of SD_1 and SD_2 phenotypes of women and their respective husbands.

The iso-immunization of the mother towards the antigens with the subsidiary classification of SD_1, SD_2 and SD_3 in the Major Histocompatibility System, is made evident by the appearance in the circulation of cytotoxic antibodies as compared with the foetus, the father and other individuals, which, while not belonging to the same family group, have antigens of histocompatibility in common.

MATERIAL AND METHODS

This study was carried out on 774 puerperae, of various parity. The maternal sera were tested for cytotovicity, by Terasaki and McClelland [1] (partially modified) test, against the husband's lymphocytes. Of 111 positive sera, the antibody characteristics were defined in 70 only, testing according to:

— Terasaki and McClelland, at room temperature [1]

— Mayer at 15 °C [2, 3] (only in 12 sera out of 70)

and against a panel of cells with known SD_1, SD_2 specificity:

— series SD_1: 1, 2, 3, 9, 10, 11, W28, W 32, W 39;

— series SD_2: 5, 7, 8, 12, 13, W5, W18, W21, W22, W14, W15, W16, W17, W27, W10.

The investigation was completed by carrying out tissue typing, in the women and their respective husbands, in 63 out of the 70 sera studied, using the method of Terasaki and McClelland [1].

The antisera used were capable of defining the specificity of the SD_1 and SD_2 series indicated above.

RESULTS

Cytotoxic antibodies and number of pregnancies

The distribution of the lymphocytotoxic sera, in relation to parity, is shown in Table 1.

Comparison of the percentages of cytotoxic antibodies present in the primiparae and those from the pluriparae, considered in aggregate, did not give a statistically significant result ($X^2 = 0.48$).

Table 1. *Ratio between no. of pregnancies and frequency of leucocytotoxic antibodies.*

No. of pregnancies	No. of pregnant women	No. of preg. women with antibodies	No. of preg. women without antibodies	% of isoimmunization	X^2
1	220	28	192	12,7	7,74
2	283	35	248	12,3	8,8
3	154	19	135	12,3	6,73
4	78	21	57	26,9	—
5	39	8	31	20,5	0,28
total	774	111	663	14,3	
primiparae	220	28	192	12,7	0,48
multiparae	554	63	471	14,9	

Antibody specificity of lymphocytotoxic sera

The specificities of the sera tested, at room temperature, are summarized in Table 2.

The antibody specificities demonstrated at room temperature and those found at 15 °C are compared in Table 3.

The 12 sera examined may be subdivided into 3 groups:

— the first group (sera 223/2, 248/2) shows partial specificity at room temperature and complete or almost complete specificity with any extra-reaction, at 15 °C;

— the second group (sera 201/2, 161/2, 254/2, 147/2, 151/2, 224/2) is made up of monospecific sera at room temperature, becoming bispecific at 15 °C;

— the third group (serum 146/2), with X and Y specificity at room temperature, gives such a wide response at 15 °C that it is not possible to define with what specificity it reacts.

Antibody specificity and parity

The relationship existing between the spectrum of antibody specificity and parity is shown in Table 4.

Theoretically, this result should be wider, on the basis of two considerations:

— because the immunizing antigens may vary from one pregnancy to another;

— because the repeated stimulations should lead to potentiation of the immune response.

It seems clear, on the other hand, that the number of antibody specificities is not substantially modified by the number of pregnancies.

Immunizing antigens and antibody specificity of the cytotoxic sera

On analysis of Table 5, the antisera can be regrouped as follows:

1) Sera with antibody specificity corresponding to the immunizing antigens (sera 1 to 19).

2) Sera with antibody specificity corresponding to immunizing antigens and to antigens cross-reacting with them (sera 20 to 31).

3) Sera that give a positive reaction to immunizing antigens, to antigens cross-reacting with them and to a certain number of extra-reactions (sera 32 to 34).

4) Sera that present positive reactions with one of the immunizing antigens and extra-reactions (sera 35 to 38).

5) Sera that react with antigens of series SD_1, SD_2, different from the immunizing antigens (sera 39 to 42).

6) Sera that present antibody specificity against antigen 4a, probably a phenotypical manifestation of an ancestral gene from which its SD_2 antigens are derived (sera 43 and 44).

7) Sera that react with the majority of the cells in the panel (polyspecific sera 45 to 54).

8) Sera that give a positive reaction with a limited number of the cells of the panel, but without defining known SD_1, SD_2 specificity (sera 55 to 61).

9) Finally, serum 62, gives a positive reaction with the husband's cells, but gave a negative result on repeated testing with the cells of the panel.

Table 2. *Sera with one specifity*

		Sera	Antiges	+/+	+/−	−/+	−/−	Total
1	S.M.A.	1.1	4a	36	1	1	14	52
2	A.M.	2.1	12	6	0	0	45	51
3	R.L.	146.2	W18	5	0	1	39	45
4	D.N.M.	147.2	7	6	0	0	39	45
5	L.G.	150.2	1	13	4	0	28	45
6	R.A.	248.2	9	28	5	0	63	96
7	C.C.	161.2	1	5	0	2	33	40
8	S.N.	129.3	2	15	0	1	34	50
9	D.C.	151.3	5	9	3	0	38	50
10	C.A.	184.2	4a	38	4	0	15	57
11	T.V.	185.2	9	21	0	0	36	57
12	E.V.	224.2	W26	5	1	0	51	57
13	B.L.	275.2	A2	8	5	0	33	46
14	S.D.	213.2	2	19	1	0	37	57
15	C.M.L.	205.2	2	19	2	0	36	57
16	S.A.	201.2	5	13	2	0	42	57
17	C.D.	212.2	W5	10	3	3	41	57
18	Z.E.	245.2	5+52	34	5	0	57	96
	Z.E.	245.2	5	32	0	0	64	96
19	T.E.	144.2	W5	12	7	0	26	45
20	D.A.G.	233.2	5	32	9	0	55	96
	D.A.G.	233.2	5	31	1	1	63	96
21	C.D.	253.3	12	7	0	1	38	46
22	B.G.	274.2	13	3	0	0	43	46
23	R.G.	254.3	1	8	0	1	37	46
24	N.O.	223.2	A2	23	0	20	53	96
25	D.P.A.	210.2	A2	27	6	16	47	96
26	G.G.	234.2	A5	11	0	21	64	96
27	S.F.	251.2	A2	12	3	21	59	95
Sera with two specificities								
28	B.G.	180.2	7+22	9	1	0	30	40
29	M.E.	211.2	10+8	19	0	1	37	57
30	S.P.	243.2	13+W10	10	0	0	86	96
31	F.T.	276.2	2+12	16	4	1	25	46
32	T.A.	272.2	9+5	18	4	5	19	46
33	B.L.	247.2	2+W28	49	7	0	40	96
34	V.L.	202.2	5+W21	16	3	0	38	57
35	F.G.	170.3	10+AJ	11	0	0	29	40
36	G.G.	168.2	12+4	8	0	0	32	40

Table 2. *(Cont.).*

	Sera	Antiges	+/+	+/−	−/+	−/−	Total
37 P.R.	127.2	2+7	19	0	0	21	40
38 S.G.	249.2	W18+8	23	6	3	64	96
39 Z.A.	222.2	11+3	25	13	3	55	96
40 P.P.	278.2	2+28	17	0	9	20	46
41 G.M.	261.2	5+W5	25	5	3	13	46
Sera with three specificities							
42 M.A.	277.2	2+5+21	16	2	3	25	46
43 M.I.	148.2	5+W5+18	25	2	0	18	45
44 F.M.	204.2	7+27+15	10	8	0	39	57
45 G.M.	241.2	2+28+W5	59	8	1	18	96
46 S.M.G.	257.2	2+28+7	21	7	1	17	46
47 C.R.	271.2	2+28+W5	25	5	1	15	46
Sera with four specificities							
48 B.D.	209.2	2+11+28+17	30	6	1	19	57
49 R.G.	160.2	2+7+22+27	23	3	0	14	40
Polyspecific sera							
50 R.C.	361		37	positive on 51 cells			
51 S.A.	4.2		44	» » 51 »			
52 L.D.	128.2		46	» » 51 »			
53 B.G.	137.2		40	» » 45 »			
54 B.L.	149.2		23	» » 45 »			
55 G.F.	181.2		34	» » 40 »			
56 B.L.	159.2		10	» » 40 »			
57 P.G.	203.2		38	» » 57 »			
58 R.G.	214.2		45	» » 57 »			
59 P.C.	237.2		24	» » 96 »			
60 D.G.	246.2		82	» » 96 »			
61 S.A.	244.2		78	» » 96 »			
62 P.F.	256.2		32	» » 46 »			
63 M.M.	279.2		75	» » 95 »			
Sera that react with a limited number of cell of the panel							
64 D.D.A.	259.2		2	positive on 46 cells			
65 Z.F.	280.2		2	» » 46 »			
66 B.G.	258.2		4	» » 46 »			
67 A.A.	260.3		4	» » 46 »			
68 B.M.	252.2		7	» » 46 »			
69 M.F.	255.2		5	» » 46 »			
70 F.A.	242.2		many reactions doubtful				
71 G.F.	167.3		1+9+10+11+30+31				

Table 3. *Comparison between antibody specificities at room temperature (15° C)*

	Sera	Antigens	+/+	+/−	−/+	−/−	Tot.	+/+	+/−	−/+	−/−	Tot.
C.C.	161.2	1	5	0	2	33	40	1	9	0	28	38
L.G.	150.2	1	13	4	0	28	40	0	0	1	37	38
S.A.	201.2	5	13	2	0	42	57	10	10	0	18	38
B.D.	224.2	W26	5	1	0	51	57	1	4	0	33	38
D.N.	147.2	7	6	0	0	39	45	8	4	0	26	38
R.L.	146.2	W18	2	0	1	37	40	Multispecific				38
T.V.	185.2	9	21	0	0	36	57	10	2	0	26	38
R.A.	248.2	9	28	5	0	63	96	10	8	0	20	38
R.G.	254.2	1	9	0	0	37	46	1	8	0	29	38
B.G.	274.2	13	3	0	0	43	46	2	0	0	36	38
D.C.	151.2	5	11	4	0	25	40	10	7	0	21	38
N.O.	223.2	2	23	0	20	53	96	12	1	4	21	38

+/+ = positive reaction of antiserum with specificity indicated.
+/— = positive reaction of antiserum with specificity different from that indicated.
—/+ = negative reaction of antiserum with specificity different from that indicated.
—/— = negative reaction of antiserum with specificity indicated.

Table 4. *Relations between parity and number of antibody specificities.*

No. of pregnancies	Women with antibodies	Number specificitiy				
		1	2	3	4	>4
1	15	7 = 46%	2	1		5 = 33,3%
2	31	10 = 32,2%	6 = 19,3%	4 = 12,9%	1	10 = 32,2%
3	12	4 = 33,3%	2 = 16,6%	1		5 = 41,6%
4	10	5 = 50%	3		1	1
5	2	1	1			
totale	70	27 = 38,5%	14 = 20%	6 = 8,5 %	2 = 2,8%	21 = 30%

The letters X and Y indicate the possible antigens of the 1st and 2nd series that were not identified, either because the antisera available to us were not able to define them, or because a homozygotic condition was concealed and could not be found without studying the families.

DISCUSSION

The results obtained provide a starting point for consideration of several points:

1) Pregnancy is an important cause of leucocytic iso-immunization.

2) Leucocytic iso-immunization may appear either in primiparae or pluriparae.

3) The lymphocytotoxic sera produced in materno-foetal immunization may present various antibody specificities.

4) The antibody specificity spectrum may vary in relation to the temperature at which the reaction takes place.

5) The antibody specificity spectrum does not seem to be influenced by the number of pregnancies.

6) The antibody specificities obtained do not always correspond to the specificities expected on the basis of the SD_1, SD_2 phenotypes of the women and their respective husbands.

Table 5. *Phenotypes SD_1 and SD_2 in women and their respective husbands: possible immunizing antigens and antibody specificities*

		HL-A antigens of wives		HL-A antigens of husbands		Immunizing antigens		Antibody specificities
1	R.L.	2-X_1	Y_1-Y_2	2-X_2	W18-W5	X_2	W18-W5	W18
2	D.N.M.	2-X_1	W18-W5	W28-W32	7-W18	W28-W32	7	7
3	D.C.	2-X_1	Y_1-Y_2	2-X_2	5-Y_3	X_2	5-Y_3	5
4	G.G.	10-X_1	Y_1-Y_2	2-X_2	12-Y_3	2-X_2	12-Y_3	12(+4a)
5	M.B.	2-9	12-Y_1	1-10	8-Y_2	1-10	8-Y_2	10-8
6	D.P.A.	3-X_1	13-Y	1-2	W5-W17	1-2	W5-W17	2-W17
7	S.D.	1-X_1	12-Y	2-X_2	W5-W14	2-X_2	W5-W14	2
8	L.G.	2-X_1	12-Y_1	1-3	8-Y_2	1-3	8-Y_2	1
9	S.A.	10-11	12-W5	2-X_1	5-12	2-X_1	(5)	5
10	N.O.	3-9	W5-W18	2-W26	7-W18	2-W26	7	2
11	C.M.	10-11	W5-Y_1	2-11	Y_2-Y_3	2	Y_2-Y_3	2
12	E.D.	1-X_1	W5-W12	10-11	W17-Y_1	10-11	Y_1	W26
13	C.B.	9-X_1	W17-W27	W26-X_2	W5-Y_1	W26-X_2	W5-Y_1	W5
14	C.D.	W32-X	12-W17	9-11	W5-W22	9-11	W5-W22	W5
15	S.F.	3-X_1	12-W18	2-X_2	5-Y_1	2-X_2	5-Y_1	2
16	R.G.	2-9	5-7	1-X_2	W5-8	1-X_2	8-(W5)	1
17	F.M.T.	1-3	5-Y_1	1-2	12-Y_2	2	12-Y_2	2+12
18	B.L.	3-W29	W21-Y_1	2-W32	7-W18	2-W32	7-W18	2
19	B.G.	W32-W25	W18-W10	1-X_1	13-W22	1-X_1	13-W22	13
20	M.I.	1-X_1	13-Y_1	1-2	W5-W18	2	W5-W18	5+W5+W18
21	Z.A.	2-W32	5-W18	11-X_1	W5-W17	11-X_1	W17	11+3
22	S.N.	1-9	5-W18	2-X_1	5-W22	2-X_1	W22	2+W28
23	G.M.	1-X_1	8-Y_1	2-X_2	5-Y_2	2-X_2	5-Y_2	2+W28+W5
24	B.L.	W32-X_1	5-Y_1	1-2	W17-W22	1-2	W17-W22	2+W28
25	S.M.G.	3-X_1	W16-Y_1	2-X_2	5-7	2-X_2	5-7	2+W28+7
26	C.R.	1-X_1	7-W15	2-X_2	5-W14	2-X_1	5-W14	2+W28+W5
27	S.G.	2-9	W17-Y_1	10-X_1	7-W18	10-X_1	7-W18	W18+8
28	B.G.	2-X_1	12-Y_1	1-2	5-7	1	5-7	7+W22
29	P.P.	3-W32	W27-Y_1	2-X_1	7-W25	2-X_1	(7)-W25	2+W28
30	B.D.	10-X_1	7-Y_1	1-2	9-2	1-2	Y_2-Y_3	2+11+W28+W17
31	G.M.	W28-W26	W14-X_1	2-9	5-W18	Y_2-Y_3	5-W18	5+W5

Table 5. (*Cont.*)

		HL-A antigens of wives		HL-A antigens of husbands		Immunizing antigens		Antibody specificities
32	F.M.A.	1-X_1	Y_1-Y_2	1-2	7-Y_3	2	7-Y_3	7+W27+W15
33	R.G.	3-10	W5-W18	2-X_1	Y_1-Y_2	2-X_1	Y_1-Y_2	2+7+W22+W27
34	T.A.	2-11	W5-W10	1-9	W21-W17	1-9	W21-W17	5-9
35	V.L.	1-X_1	7-Y_1	1-X_2	5-Y_2	X_2	5-Y_2	5+W21
36	S.G.	3-X_1	7-12	9-10	Y_1-Y_2	9-10	Y_1-Y_2	10+AJ
37	S.P.	9-11	W18-Y_1	X_1-X_2	W10-W22	X_1-X_2	W10-W22	13+W10
38	Z.E.	2-11	W10-Y_1	2-W32	5-W17	W32	5-W17	5
39	G.G.	2-11	12-W5	2-X_1	W5-Y_1	X_1	Y_1	5
40	B.M.G.	1-2	W5-Y_1	1-X_1	W18-Y_2	X_1	W18-Y_2	5+extra reac.
41	R.A.	2-10	5-Y_1	W28-X_1	5-W22	W28-X_1	W22	9
42	C.D.	1-9	W5-W27	11-X_1	Y_1	11-X_1	Y_1	12
43	C.A.	1-X_1	W14-Y_1	X_2-X_3	W17-W21	X_2-X_3	W17-W21	4a
44	S.M.A.	2-11	W5-W18	3-W32	5-Y_1	3-W32	5-Y_1	4a
45	P.F.	1-2	12-Y_1	2-10	5-Y_1	10	5-Y_1	polispecific
46	S.A.	1-3	5-W27	1-2	8-W22	2	8-W22	»
47	D.D.G.	9-X_1	7-W14	1-3	7-Y_1	1-3	Y_1	»
48	P.G.	10-X_1	12-Y_1	11-W32	W5-Y_2	11-W32	Y_2	»
49	P.M.	11-X_1	5-Y_1	2-3	W10-Y_2	2-3	W10-Y_2	»
50	S.A.	9-X_1	7-W14	2-X_2	5-Y_1	2-X_2	5-Y_1	»
51	R.G.	1-2	8-W17	2-3	5-W17	3	Y_1	»
52	G.F.	2-3	12-W5	1-9	W5-Y_1	1-9	5-Y_2	»
53	B.L.	1-X_1	W5-Y_1	1-2	W5-Y_2	2	5	»
54	M.M.	2-W29	12-Y_1	3-11	5-Y_2	3-11	Y_2	»
55	D.D.A.	2-3	5-W18	2-X_1	W5-Y_2	X_1	7-Y_1	2 pos. su 46 cells
56	Z.F.	2-3	W5-W10	3-X_1	7-Y_1	X_1	5-Y_1	2 » » » »
57	B.G.	2-9	W17-W21	2-X_1	5-Y_1	X_1	W21-Y_1	4 » » » »
58	A.A.	1-2	8-W5	11-Y_1	W21-Y_1	11-X_1	W5-12	4 » » » »
59	B.M.	2-3	7-W16	3-11	W5-12	11	7-W5	7 » » » »
60	M.F.	2-11	W14-W18	3-W32	7-W5	3-W32	Y_2	5 » » » »
61	F.A.	3-X_1	W5-Y_1	3-9	5-Y_2	9	7-Y_3	reactions doubtful
62	M.E.	2-X_1	Y_1-Y_2	2-9	7-Y_3	9	W5-Y_2	

CONCLUSIONS

Supported by other authors ([4,5,6,7]), we can state that iso-immunization of the mother, as compared with the antigens of foetal incompatibility, can fundamentally appear in 5 ways:

1) With the production of an antiserum, in which all the expected antibody specificities are present.

2) With the production of an antiserum in which only some of the expected antibody specificities are present.

3) With the production of an antiserum with antibody specificity in greater numbers than those corresponding to the immunizing antigens.

4) With the production of antisera with antibody specificities in such number that it is not possible to define them.

5) With the production of antisera that react with a limited number of cells without any of the known SD_1, SD_2 specificities being disclosed.

We consider ([8,9]) that the definition of antibody specificity of these sera represents the keystone through which at least some of the many problems can be explained that still exist around the structure of the Major Histocompatibility System in man and concerning the complex immunological interactions between mother and foetus during pregnancy.

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BIBLIOGRAPHY

1) Terasaki P.I., McClelland J.D.: *Nature*, 204, 998, 1967.

2) Mayer S., Falkendrot A., Tongio M.M.: *Tissue Antigens, 3,* 431, 1973.

3) Mayer S., Falkenrodt A., Tongio M.M.: *Tissue Antigens, 3,* 387, 1974.

4) Staub-Nielsen L., Swejgaard A.: *Tissue Antigens, 2,* 316, 1972.

5) Ceppellini R.: *Min. Gin., 20,* 857, 1968.

6) Palm J.: *Transplant. Proc., 2,* 162, 1970.

7) Bach F.H.: *La Ricerca, 3,* 527, 1974.

8) Ahrons: Personal Comunication, 1970.

9) Van Rood J.J., Bernisse J.G., Leeuwen A.: *Nature, 181,* 1735, 1959.