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CELLULAR IMMUNITY TO PENICILLIN IN NEWBORNS

By

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It has previously been shown that the leukocyte migration test (LMT) is a useful method for the study of cellular immunity (CI) to penicillin (BP) in man (8, 9).

By means of this technique CI to BP has been demonstrated in 26 per cent of normal non-allergic subjects tested at least 1 year after a treatment by BP. Cellular immunity is thus not uncommon in a normal adult population (5). The frequency of this sensitization may be related to the high rate of therapeutic and non-therapeutic exposures of humans to penicillins (3, 10).

At what stage young children acquire a sensitization to penicillin has not been documented but such a sensitization is known to sometimes occur (4).

In this study we have looked for the existence of a cellular immunity to penicillin in newborns and its induction by a penicillin treatment at birth.

MATERIAL AND METHODS

Newborns

Thirty newborns with normal levels of blood lymphocytes and immunoglobulins G, A and M were studied. They fell into two groups as follows:

(1) Eleven neonates untreated with penicillin; nor had the mothers been treated with penicillin during pregnancy. These newborns, (gestational age 40 weeks) had bacteriological blood monitoring for suspicion of neonatal infections (confirmed only once). Blood was obtained by venipuncture on the 4th day of life (range 1-12).

(2) Nineteen newborns (gestational mean age 34 weeks range 29-42) were treated with penicillin (100,000 iu/kg/24 h) or Ampicillin (200 mg/kg/24 h) in an intensive care unit during 9 days (range 7-16). The diseases were: idiopathic respiratory distress syndrome (12); streptococcal infections (2); meconium aspiration (1); foetal distress (1); diaphragmatic hernia (1); congenital stridor (1); suspicion of foetopathy (1). Blood was collected either by venipuncture or from umbilical catheter on the 12th day of life.

Leukocyte Migration Test in Newborns

The technique, very similar to that of Søborg & Bendixen (11) has been previously described (6).

Modifications for the use of this technique in newborns were as follows:

3 to 5 ml of blood were collected on preservative-free heparin (50 iu/ml). The spontaneous sedimentation at 37° C lasted 1 to 2 h in a plastic syringe. Leukocytes were washed three times in Hanks's solution at 37° C. The suspension was adjusted to 5×10^7 cells/ml in TC 199 containing 10 per cent horse serum. The suspension was drawn into three μ l-microcapillary tubes (Drummond) sealed with paraffin and centrifuged at 1800 r.p.m. for 5 min. The microcapillaries were then cut 0.5 mm above the cell-fluid interface and fixed on the sterile glass siliconized culture chambers with silicone wax, two microcaps per chamber. The control migrations (six) were performed with TC 199 containing 10 per cent horse serum and the test migrations (four for each concentration of antigen) were performed with freshly prepared benzyl penicillin (1000, 5000, 10,000 iu/ml) in TC 199 + 10 per cent horse serum.

The migration areas were measured by planimetry.

The interpretation that a penicillin-induced inhibition reflects a positive test has been previously discussed (7).

Briefly: A 0.80 migration index was considered to be the highest value for a positive test, as it has been shown by numerous authors to be the limit of a significant inhibition.

The significance of each inhibition was further assessed as follows: for each control migration serie, the mean (MC) and the standard deviation (s.d.) were calculated; in each experiment reported as positive, the mean of migration values with penicillin (MP) was: $MP \leq MC \pm 3 \text{ s.d.}$ (i.e. a significance at the 0.01 level).

In some cases a stimulation of the migration was observed. This probably reflects a specific phenomenon (13, 14). It was considered significant when $MP \geq MC \pm 3 \text{ s.d.}$

Parallel Studies

In order to see whether the leukocyte migration test with 3 μ l microcapillaries was reflecting the same phenomenon as the test with 20 μ l microcapillaries, 26 parallel tests were performed with the leukocytes of adults packed in 3 μ l

and 20 μ l microcaps. These 26 subjects were known to have either a positive or a negative leukocyte migration test to penicillin. The same suspension of leukocytes (prepared as previously described for newborns) was drawn either into 20 or 3 μ l microcapillaries and the migrations were performed in the presence of penicillin (1000, 5000, 10,000 iu/ml).

TABLE 1

Comparison between the MI of Adults' Leukocytes Packed in 20 μ l Microcapillaries and in 3 μ l Microcapillaries.

Penicillin 1000 iu		Penicillin 5000 iu		Penicillin 10,000 iu	
20 μ l	3 μ l	20 μ l	3 μ l	20 μ l	3 μ l
0.91	0.98	—	—	0.88	1.10
0.91	0.98	1.14	0.80	1.17	1.04
1.07	0.88	1.09	1.09	0.99	1.01
1.02	1.02	1.07	1.11	1.09	0.95
0.97	0.90	—	—	0.98	0.69*
1.04	0.93	—	—	1.15	0.96
—	—	—	—	0.98	0.92
—	—	1.05	1.13	1.14	1.13
—	—	1.00	1.17	1.03	1.04
—	—	0.84	0.96	1.08	0.94
—	1.04	0.85	1.00	0.86	0.91
—	0.98	—	0.91	0.94	1.01
—	1.12	1.06	1.11	0.92	1.09
0.98	0.98	—	1.03	0.98	0.99
1.01	0.81	—	0.86	0.90	0.82
0.94	1.07	1.03	1.00	0.82	0.96
0.82	1.01	0.86	0.95	0.72	0.79
0.89	1.02	—	—	0.71	1.00*
1.00	1.00	—	—	0.77	0.57
0.83	0.88	0.64	0.60	0.63	0.58
—	—	—	—	0.72	1.08*
0.57	0.46	0.79	0.44	0.46	0.48
0.96	0.70*	0.92	0.91	0.72	0.79
0.89	0.91	0.76	0.79	0.61	0.78
1.05	0.98	0.82	0.96	0.80	0.74

Out of 58 paired experiments there are only four different results*. Two positive results with 3 μ l but negative with 20 μ l. Two negative with 3 μ l but positive with 20 μ l.

(Positive results in italics).

RESULTS

Parallel Study in Adults

Table 1 shows that the leukocyte migration test gave very similar results with either 20 μ l or 3 μ l capillaries.

TABLE 2
Leukocyte Migration Test (LMT) in 3 μ l Microcapillaries.

Non-treated newborns				Treated newborns			
No.	Penicillin 1000 iu	Penicillin 5000 iu	Penicillin 10,000 iu	No.	Penicillin 1000 iu	Penicillin 5000 iu	Penicillin 10,000 iu
1	0.86	1.00	—	12	0.93	1.05	
2	0.92	0.87		13	0.95	1.04	
3	1.01	0.84		14 R	1.24	0.82	
4	1.29	1.07		15	1.01	0.88	
5	1.19	1.00		16	1.00	1.02	
6	1.18	1.09		17	0.92	0.76	
7	1.11	1.01		18 R	1.14	1.37	
8	1.10	0.91	0.94	19 R	0.83	0.76	
9	1.08	1.09		20	1.03	1.01	
10	1.05	1.09	1.21	21	1.07	0.89	
11	0.87	1.08		22	0.94	1.02	
				23	1.10	1.11	1.16
				24 R	0.97	0.93	0.81
				25	0.85	0.93	0.95
				26	0.97	0.98	0.89
				27	0.95	0.94	0.99
				28	1.08	1.09	1.21
				29	1.11	0.93	0.91
				30	0.96	1.08	0.84

Results are given as migration indexes. There is no positive LMT with penicillin in the newborns who have not been treated with penicillins. There are three positive LMT in the group of newborns treated with penicillins.

R: maculopapular skin rash during the treatment.

(Positive results in italics).

Newborns

Table 2 shows that the LMT was always found to be negative in the newborns who had not been treated with penicillin. In the group of newborns treated with penicillin three LMT

were found to be positive. A maculopapular skin rash had occurred during the treatment in four babies; two of them had a positive LMT.

DISCUSSION

The aim of this work was to study the cellular immunity (CI) to penicillin (BP) in newborns by means of the leukocyte migration test. For this purpose it was necessary to use a micro-method. We have clearly shown that 3 μ l microcapillaries could be used instead of 20 μ l microcapillaries, the results being very similar in pair experiments using 3 μ l and 20 μ l microcapillaries. Therefore it was possible to use the leukocyte migration test (LMT) in newborns. This had been previously established by Fimmel (1).

There was no detectable CI to penicillin in newborns before any treatment with penicillin. This fact may suggest that non-therapeutic penicillins in the environment of the mothers (3) were unable to sensitize the foetuses. It is indeed well established that the foetus can develop an immune response to antigens which cross the placental barrier (2, 12).

A cellular immunity to BP has been detected in three of 19 newborns treated with penicillin. This result suggests that the treatment has actually induced the CI. The proportion of newborns who became sensitized (15 per cent) is lower than the proportion found in an adult population (50 per cent) (5); this may reflect the fact that in adults, it is most probably a secondary immune response instead of a primary one which is supposed to occur in newborns. Nevertheless, our results show that the induction of a cellular immunity to BP may take place very early in life.

A maculopapular rash was observed during the treatment in two of the three newborns who developed a CI to penicillin. As no supporting evidence for any other aetiology has been found to explain this rash it could be considered to be a clinical manifestation of sensitization to penicillin.

SUMMARY

A leukocyte migration micromethod has been used to study the cellular immunity to penicillin (CIP) in newborns. No detectable CIP has been found in newborns who had not been treated with penicillin. Fifteen per cent of the newborns treated with penicillin were found to have a CIP as early as the 12th day of life.

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