through radio and TV. Radio can be made to provide two-way communication, giving women the chance to talk about some of their concerns.

Support from husbands and families was also an important factor in deciding to be tested. Traditionally, in Tanzanian society, women cannot voice their individual opinions.[21] As shown in Figure 5, 60.9% of women answered that their husbands were the key persons to decide important domestic issues, while 25.5% of women answered that household decisions were made mutually. It suggested that the power and gender relations in this cultural setting are generally in favor of men, and that women tend to conceal matters that may put their relationships with men in jeopardy.[22] Moreover, they are fearful of violence, divorce, and of being left alone to die if they are found to be infected with HIV.[7,23–28] Women need to consult their husbands before getting tested, which is one of the reasons for refusing HIV testing. Although 70.0% of those who were tested answered that they decided by themselves, the PMTCT program should be extended to husband or partners.

In the univariate analysis, lack of community support, privacy and confidentiality of counselors were responsible for the low acceptance of testing. Pregnant women were afraid of being stigmatized or discriminated against in the community as well as being shunned by family.[7,17,29] Bunting noted that the main causes of this fear were related to insufficient and inaccurate knowledge, fears of death and disease, sexual norms and a lack of recognition of stigma.[30] Knowledge of HIV/AIDS and MTCT of HIV should also be expanded to the community for reducing ignorance and prejudice, and HIV counseling plays an important role in reducing women's fear of and stigma associated with the testing.[31,32]

The present study has several limitations. First, the study was done in only the urban area. In Tanzania as well as other developing countries, rural living conditions are much more difficult than that of urban areas. Stigma and discrimination leading to denial of HIV may be deep-rooted, and agreeing to HIV testing may be more stressful to women living in the local setting. Further research will be needed, especially in rural areas, in order to grasp the whole situation in Tanzania. Second, the study needs to focus more on the counseling aspect of the PMTCT program. Health-care providers play an important role in increasing acceptance of the program and in maintaining participant privacy. Many counselors' training courses were held under the auspices of the MOH and other organizations including non-government organizations in the region. However, the qualities of HIV counseling in the study area are still questionable. Considering these situations, further studies are needed for the successful expansion of the PMTCT program and to ensure the best possible outcome for both HIV-positive pregnant women and their children.

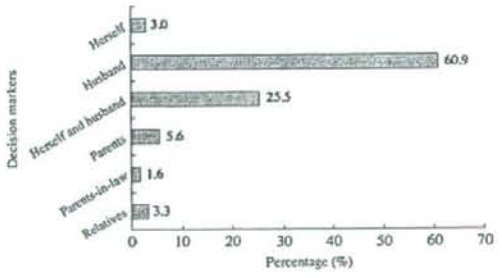

Fig. 5 Final decision on domestic issues.

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Feature Article

Nutritional status of low-birthweight ethnic minority infants in Backan province, Vietnam

NGUYEN THANH HIEN AND HIROSHI USHIJIMA

Department of Developmental Medical Sciences, Institute of International Health, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan

Abstract

Background: Birthweight and length have been reported to be important determinants of infant growth and future nutritional status. The study aims to describe the weight and length growth patterns during the first year of life of low-birthweight (LBW) ethnic minority infants in the mountainous province Backan, Vietnam.

Methods: A total of 64 LBW and normal birthweight infants of ethnic minority mothers were recruited from 2001 to 2002 into a prospective cohort study. The weight and length of infants were measured monthly for 1 year. Data on nutritional status and feeding practices of the infants were collected from monthly health records and face-to-face interviews with mothers while their infants were 6 and 12 months of age.

Results: Most of the increase in weight, length and catch-up to the 10th percentile for LBW infants occurred during the first 3 and 6 months for boys and for girls, respectively. After these ages, the mean weight and length diverged from National Center for Health Statistics (NCHS) reference curves to below the 10th percentile. LBW infants' weight-for-age z-scores was below the NCHS standard at birth (−2.16 SD), caught up after birth, became sustainable by 4 months, fell rapidly from the sixth month, then decreased to −2 SD at 12 months of age. LBW infants' length-for-age z-scores increased in the first month after birth, decreased in the second month and sharply increased again until 5 months of age before decreasing.

Conclusions: For LBW infants, it is difficult to achieve the same weight or length curves at 12 months of age as the NCHS standard.

Key words ethnic minority, low birthweight, nutritional status, Vietnam.

Weight and length at birth have been reported to be important determinants of infant growth[1] and future nutritional status.[2] The first year of life is characterized by extremely rapid growth and most growth faltering, resulting in underweight and stunting, occurs within a relatively short period from before birth until about 2 years of age. A low-birthweight (LBW) infant is more likely to be underweight or stunted in early life.[3] These infants are known to be at risk of long-term growth deficits with little chance of fully catching up.[4] Furthermore, infants born with LBW suffer from extremely high rates of morbidity and mortality from infectious disease and are underweight, stunted or wasted beginning in the neonatal period through childhood. It has been estimated that for term infants weighing 2000–2500 g at birth, the risk of neonatal death is 10 times higher than for infants weighing 3000–3500 g.[5]

Correspondence: Hiroshi Ushijima, Department of Developmental Medical Sciences, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113–0033, Japan. Email: ushijima@m.u-tokyo.ac.jp

Many studies have sought to answer the question whether LBW infants have normal growth status compared with normal birthweight (NBW) and international standard growth recommended by the National Center for Health Statistics ([NCHS]/World Health Organization). Most studies have focused on very LBW[6] or LBW preterm infants.[7] A full understanding of the correlation between birthweight and nutritional status of LBW at term infants during the first year of age is, therefore, necessary for developing nutrition intervention strategies in early childhood.

In Vietnam, LBW and malnutrition are considered major public health problems, as the prevalence is 7.3 and 33.8%, respectively.[8] The actual LBW figure may be higher, because in some communities only a small proportion of infants are born in a health-care facility where birthweight can be measured.

Since no specific studies about nutritional growth of LBW infants during the first year of life have been conducted in Backan, this paper is to describe the weight and length growth patterns during the first year of life of LBW at term ethnic minority infants compared with NCHS/WHO standard growth

curves; and to evaluate the nutritional status of LBW at term infants in the first year of life.

Methods

Sample and data collection

A prospective cohort study was conducted in Backan, a mountainous province in the north of Vietnam, about 160 km from the capital, Hanoi. Its population is 280 697, and 80% inhabitants belong to ethnic minority groups. A total of 35% of children under 5 years of age suffer from malnutrition, and 8% of infants are born with a birthweight less than 2500 g.[9]

Study infants were recruited from 20 health centers and the provincial hospital over a 1-year period from August 2001 to July 2002. The research sample consisted of 32 ethnic minority mothers of LBW infants and 32 control mothers who had a NBW infant. Each mother of a LBW infant was matched by age and infant's gender to the next eligible mother with a NBW infant. Criteria for sample selection were: singleton birth at term (gestational age ≥37 weeks), no congenital anomaly, and weight at birth 1500–2499 g for the LBW and 3000–3500 g for the NBW infant group.

The infants of the mothers who were agreed to enter the study were followed up from August 2001 to July 2003. Infants' weights and lengths were measured at birth and about every 30 days from birth to 12 months of age. Other information, such as duration of exclusive breastfeeding and feeding patterns, as well as infant morbidity, was based on a monthly child health self record and questionnaire interview at 6 and 12 months of age for mothers.

Athropometry

The weight and length of infants were measured monthly at time of birth to 12 months of corrected age by trained health workers at health centers, during home visits at birth and subsequently. Infants with light clothing were weighed to the nearest 10 g with a portable infant scale and the supine length was measured to the nearest 0.1 cm using a wooden length board.[10] These measurement tools were recommended by the Vietnamese National Malnutrition Prevention program. Infant weights and lengths were converted into a weight-for-age z-score (WAZ) and a length-for-age z-score (LAZ) using median values from the NCHS as the reference.[11]

Data analysis

Infants were excluded from the study if they were twin births or had any congenital anomalies likely to affect birthweight and subsequent growth. Infants were also excluded if their gestational age at birth was less than 37 weeks. Only data of infants whose growth data during the first year of life were complete were used for analysis in the study.

LBW at term infants were defined as infants whose gestational age was ≥37 weeks and whose weight at birth was <2500 g. Weight and length gains were used as indicators to assess the infant's physical growth. Weight/length gain in relation to birthweight/length was defined as differences between the measurement at the 12th month minus the measurement at birth and divided by the weight/length at birth.[12] Weight and length-for-age z-scores at 12 months of age compared with at birth were used to evaluate infants' improvement and catch-up. Z-score catch-up was defined as change above −2 SD (22 catch-up growth). Stunting was defined as a LAZ score of less than −2 SD; underweight as WAZ score of less than −2 SD on the basis of NCHS/WHO reference data.

All the data were analyzed by using Statistical Package for the Social Sciences (SPSS version 10.0) program for Windows. Standardized LAZ score and WAZ score were calculated with the EPINUT program in Epi-Infor version 2000. Bodyweights and lengths at different ages were compared with the NCHS reference growth curves with 10th and 90th percentiles. Growth measurements of term infants by gender taken from the NCHS population were used for comparison. An independent Student's *t*-test was used to test for the differences in weight and length gain between the two groups. A paired samples *t*-test was used to compare means of z-scores at birth and at 12 months of age. A *P*-value of less than 0.05 ($P<0.05$) was adopted as the criterion of significance.

Results

Characteristics of study sample

The characteristics of the study sample are presented in Table 1. During 1 year, from August 2001 to July 2002, 64 infants were recruited into the study. All those mothers were ethnic minority farming women and the average age was approximately 23.8±4.2 years and schooling was 7.6±2.1 years. The range of birthweights was 2200–3500 g, with a mean of 2762±498 g.

Among these eligible subjects, a final sample of 20 ethnic minority LBW infants and 31 control NBW infants completed the 12-month follow-up period. Of those who dropped out, 12 LBW and one NBW infants did not complete the 1-year follow up due to neonatal death or emigration from the research areas. In total, 51 infants were monitored at gestational age around 39 weeks, with a mean birthweight of 2264±173 g in the study cases and 3226±182 g in the control group. Length at birth was 45.8±1.2 and 49.0±1.2 cm for LBW and NBW infants, respectively.

Table 1 Characteristic of study sample

	LBW (n=20)	NBW (n=31)	$P^{\dagger}$
Weight at birth (g)	2264±174	3226±183	<0.001
Length at birth (cm)	45.8±1.2	49.0±1.2	<0.001
Gestational age (weeks)	39.4±3.0	39.5±1.9	0.81
Exclusively breast-fed (months)	3.05±0.76	3.32±0.7	0.19

†Student's t-test.
LBW, low birthweight; NBW, normal birthweight.

Weight gain

The mean birthweight was 2264±173 g in the study cases and 3226±182 g in the control group. The differences in weight indicators continued to be significant at 1 year of age with 7585 and 8869 g for LBW and NBW infants, respectively. At 12 months of age, bodyweight was 3.3 and 2.7 times weight at birth for LBW and NBW infant groups, respectively (Table 2).

Mean weight gain at 1 and 12 months of age was 133 and 321 g, respectively, higher in NBW than in LBW infants (P<0.001). In contrast, higher weight gain relative to birthweight was observed in LBW than in NBW infants. At 1 month of age, the difference was 8.6% (P=0.03) and this increased to 61.6% (P<0.001) at 12 months of age. Weight gain relative to birthweight at 12 months was significantly higher in LBW than in NBW infants (P<0.001). The mean postnatal weight gains for LBW and NBW are shown in (Table 3).

Figure 1a,b show the observed mean weights of LBW and NBW study infants by gender as they appear on standard NCHS growth curves. During the first year of age, the mean weight of both boys and girls with LBW was at the lower median level of NCHS reference growth curves. Most of the increase in weight and catch-up to the 10th percentile of LBW infants occurred during the first 3 months for boys and during the first 6 months for girls. After these ages, the mean weight diverged from the NCHS reference curves to below the 10th percentile.

Table 2 Mean weight and length at 1 and 12 months of age

	LBW (n=20)	NBW (n=31)	$P^{\dagger}$
At birth			
Weight (g)	2264±174	3226±183	<0.001
Length (cm)	45.8±1.2	49.0±1.2	<0.001
At 1 month			
Weight (g)	3258±523	4354±382	<0.001
Length (cm)	49.6±1.9	53.6±1.8	<0.001
At 12 months			
Weight (g)	7585±808	8869±1025	<0.001
Length (cm)	69.9±4.2	73.7±3.7	0.001

†Student's t-test.
LBW, low birthweight; NBW, normal birthweight.

Table 3 Weight gain of study infants

	LBW (n=20)	NBW (n=31)	$P^{\dagger}$
At 1 month (g)	995±431	1128±317	0.20
At 12 month (g)	5322±786	5643±947	0.21
Relative to birthweight at 1 month (%)	43.6±18.5	35.0±9.9	0.03
Relative to birthweight at 12 months (%)	236.5±39.4	174.9±27.6	<0.001

†Student's t-test.
LBW, low birthweight; NBW, normal birthweight.

The boys approached the 50th percentile at 3 months of corrected age and tended to decrease, whereas the girls grew along the 10th percentile between 1 and 7 months of corrected age.

The weights of the NBW infants in the first 6 months appeared normally distributed, and started to falter between the 50th and the 10th percentiles afterward.

Length gain

Length at birth was 45.8±1.2 cm for LBW and 49.0±1.2 cm for NBW infants. NBW infants achieved greater increases in length than LBW infants at 1 month (0.8 cm, P=0.11) and 12 months after birth (0.5 cm, P=0.66). Length gain relative to length at birth was 1% (P=0.32) higher at 1 month of age in NBW than in LBW infants. However, at 12 months of age, length relative to length at birth was 2.6% (P=0.26) lower in NBW infants than in LBW infants. Despite differences in length gain at 1 and 12 months of age between the two groups, neither the length-gain nor the length-gain relative to size-at-birth differences were statistically significant. Detail of length gains are displayed in Table 4.

Figure 2a,b present the growth curves of length for age of LBW and NBW infants by gender compared to the NCHS standard. For both boys and girls with NBW, their length for age from birth to 12 months of age run parallel to the NCHS median growth curve. The girls' growth curve in the LBW infants group grew along the 10th percentile curve, the boys' growth curve was parallel to and below the 10th percentile of the standard NCHS reference curve.

Nutritional status

The mean WAZ during the first year for LBW and NBW infant groups is shown in Figure 3. The trend of mean WAZ was the same in both groups during the first 12 months of age, but at a higher level in NBW infants. From birth to about 4 months, the study infants showed substantial improvement in mean WAZ

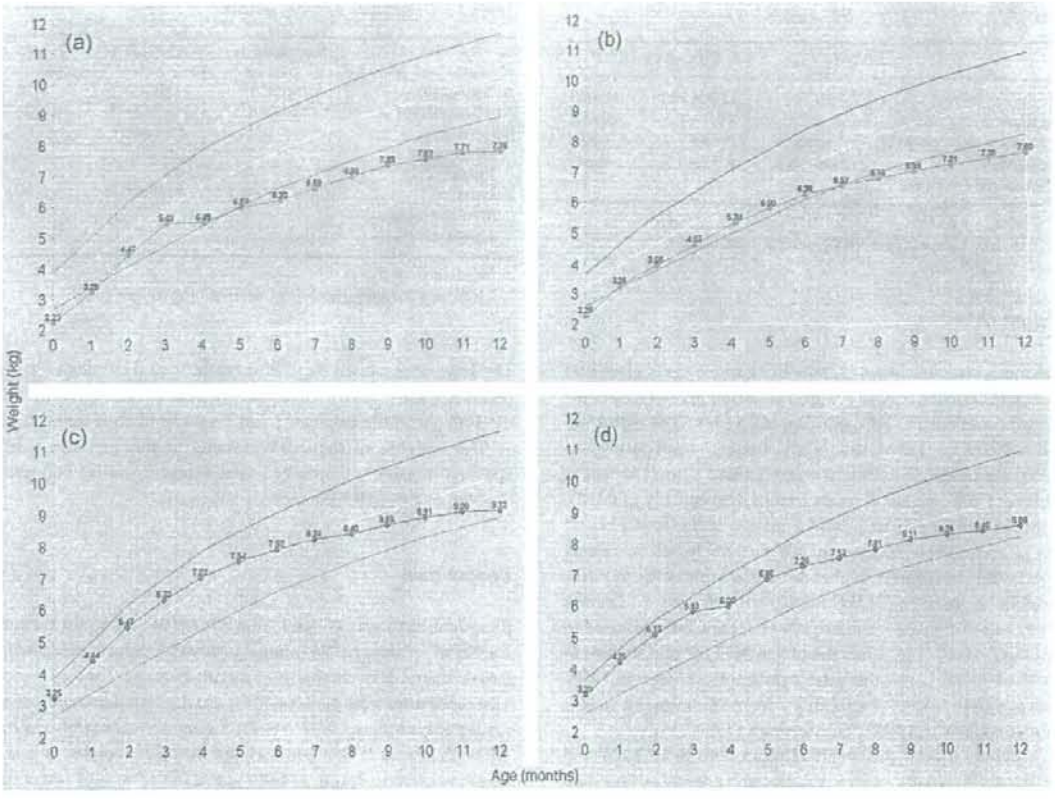

Fig. 1 (a) Mean weights of boy low birthweight and (c) normal birthweight study infants. (b) Mean weights of girl low birthweight and (d) normal birthweight study infants.

and showed early faltering after that. LBW infants had WAZ scores below the NCHS standard at birth, but catch-up after birth. At 2 months of age, mean WAZ was twice that at birth (−1 SD compared to −2.16 SD), became sustainable by 4 months, but fell rapidly from the sixth month, and declined to −2 SD at 12 months. However, WAZ at 12 months was higher than at birth ($P=0.5$). In contrast, the variation in weight gain was slightly less marked in NBW infants than in LBW infants. It was 0.49 SD at 4 months of age, and fell after that, and at 12 months of age mean WAZ was about 1 SD lower than at birth (−0.98 SD compared to −0.007 SD; $P<0.001$). At 12 months of age, 50% of LBW infants were underweight. Overall, only about one-fourth of LBW infants never had a WAZ less than −2 SD at any point during the first year of life. At some point between birth and 12 months of age, 95% of LBW infants had improved WAZ, 45% of LBW infants had improved WAZ and 60% of underweight infants achieved catch-up growth.

LAZ is considered to be a good indicator of the nutritional status and health of infants and young children. During the

Table 4 Length gain of study infants

	LBW ($n=20$)	NBW ($n=31$)	$P^{\dagger}$
At 1 month (g)	3.8±1.7	4.6±1.6	0.11
At 12 month (g)	24.2±4.2	24.7±3.4	0.66
Relative to length birth at 1 month (%)	8.3±3.9	9.3±3.4	0.32
Relative to length birth at 12 months (%)	53.0±9.4	50.4±7.0	0.26

†Student's *t*-test.
LBW, low birthweight; NBW, normal birthweight.

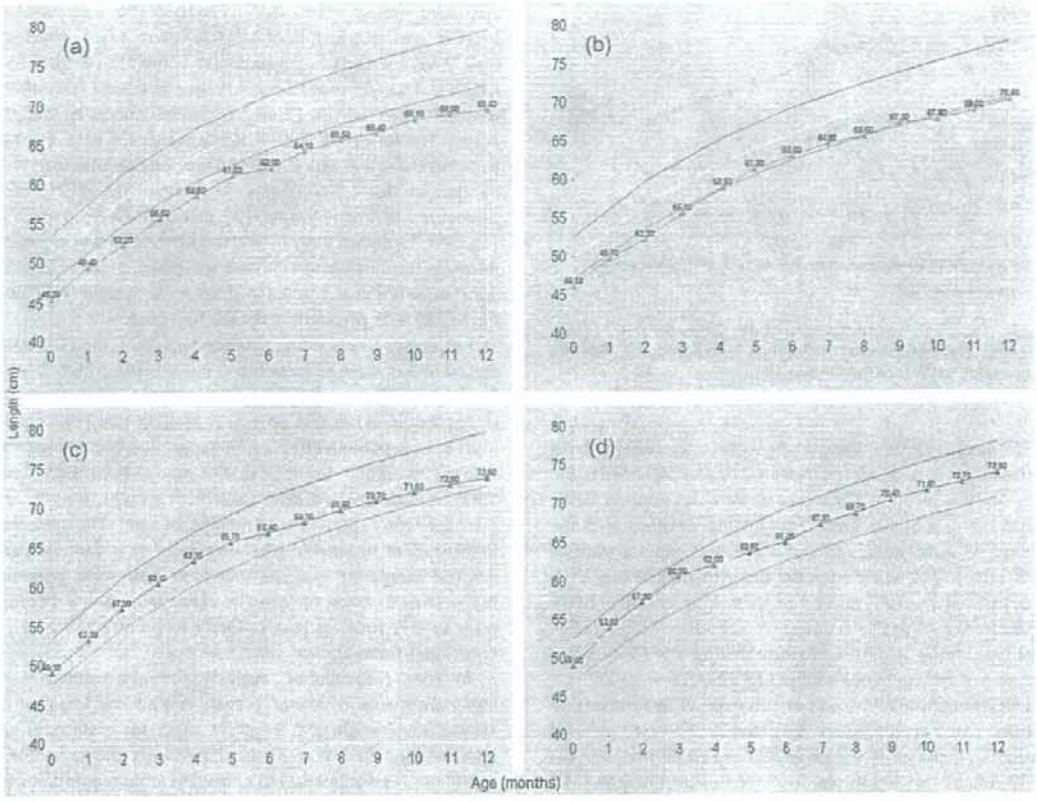

Fig. 2 (a) The growth curves of length-for-age of boy low birthweight and (c) normal birthweight infants compared to the National Center for Health Statistics standard. (b) The growth curves of length-for-age of girl low birthweight and (d) normal birthweight infants compared to the National Center for Health Statistics standard.

first year of age, LBW infants' LAZ improved: LBW infants' LAZ increased in the first month after birth and decreased in the second month and sharply increased again to 5 months of age. After that, LAZ decreased; at 12 months of age it was higher than at birth (−1.83 SD compared with −1.99 SD; $P=0.66$). NBW infants' LAZ increased after birth and decreased from 4 months of age. However, these increases were slower than in the LBW group, and LAZ at 12 months of age was lower than at birth ($P=0.36$). The trend distribution of LAZ scores during the first year are shown in Figure 4. Stunting indicates low growth and is the cumulative effect of low or inadequate intake of energy. At birth, stunting was observed in 40% of LBW infants. Among stunted LBW infants, catch-up growth sufficient to bring LAZ score above −2 SD was apparent in 62.5% at 12 months of age. Of LBW infants, 75% were stunted at least 1 month during the first year of life.

Discussion

Weight-for-age is commonly used as an indicator for malnutrition because weight is easier to measure than height. Weight-for-age reflects linear growth and weight accumulation achieved pre- and postnatal over the long term as well as weight accumulation in the short term. The results of this study demonstrate that in the first month and at 12 months of age, at term LBW infants gained weight and length more slowly than NBW infants. (These differences were insignificant.) However, a significantly higher weight growth rate was observed in LBW infants than in NBW infants by comparing the rates of weight gain at 12 months of age with respect to birthweight.

Low weight-for-age may reflect either normal variation in growth or a deficit in growth. The study sample showed that the WAZ and LAZ scores of LBW infants were below the

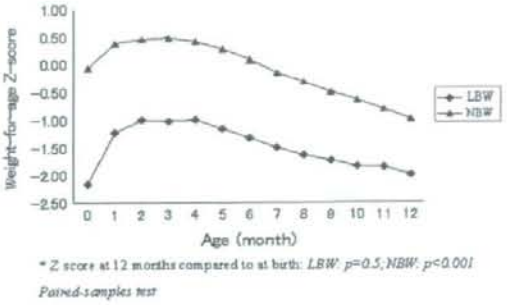

Fig. 3 The mean weight-for-age z-score during the first year for low birthweight and normal birthweight infant groups. LBW, low birthweight; NBW, normal birthweight.

NCHS median all year. The highest scores occurred after birth until 4 or 5 months of age, but fell after that and returned to the same level as at birth. These trends were the same in NBW infants but at a higher level. This finding contrasts with the findings of some studies in India, in which rapid growth of LBW infants was observed in the first 6 months of life.[13,14] A study by Sridhar *et al.*[15] showed good catch-up growth of LBW babies from 4 to 6 months of age. In several previous studies from developing countries, growth faltering was identified at 3 months of age compared with the NCHS references.[16,17]

Our comparison of z-scores at 12 months of age and at birth revealed that z-scores (mean WAZ and LAZ) were increased during the first year in the LBW infants and decreased in the NBW infants. No significant differences were found in LAZ scores at birth and at 12 months of age in either group of study infants. At 12 months of age, NBW infants had very significantly decreased WAZ scores ($P<0.001$), but a slightly significant increase was found in the LBW infants. This finding is consistent with a previous study by Brazil (1996) with full-term LBW infants, in which most LBW full-term infants improved their WAZ scores between birth and 12 months of age.[18] Our length and weight growth curve finding in these LBW infants parallel to but remain below the growth curves considered normal for infants of the NCHS references. These findings were largely in line with results in a previous longitudinal study by Badson that LBW infants never attain the NCHS 50th percentile and drop below the 10th percentile from around 5 to 6 months.[19] Another study by Wells *et al.* showed that growth of infants in the developing countries was poor compared to western standards and at 12 months of age mean weight was below the NCHS 50th percentile, even the 10th percentile.[20]

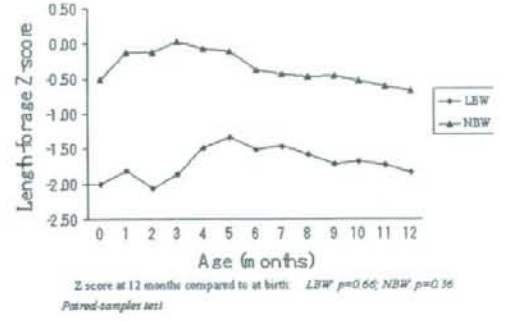

Fig. 4 The trend distribution of length-for-age z-score scores during the first year of life among study infants. LBW, low birthweight; NBW, normal birthweight.

For most infants in the developing world, the post-natal period that is most susceptible to poor linear growth begins from about 3 to 6 months and continues until about 24 to 36 months. After this time, it is thought that poor conditions have less of an effect on linear growth because growth velocity is much lower.[21] In the study, both LBW and NBW infants showed poor growth in weight, length, and z-scores from about 5 to 6 months of age. The observed improvement in nutritional status in the first 4 months and reversal thereafter suggests that there was some potential for postnatal catch-up growth. This is perhaps because prior to this time, growth is likely to be affected only by nutritional factors.

In poor communities, exclusively breast-feeding is an important factor in infants' growth. A study by Lucas *et al.* showed that weight and length of small for gestational age infants during the first year were significantly greater in breast-fed infants than in infants fed a standard term infant formula.[22] The long-term effect of breast-feeding on the nutritional status of children is also recognized in Vietnamese children with birthweight >2500 g.[23] In the present study, mean duration of exclusive breast-feeding was approximately 3 months after birth (Table 1). At 12 months of age all study infants were still breast-fed. The gradual introduction of supplementary food into the diet of study infants from around the fourth or fifth month postpartum could contribute to the explanation of poor growth after this age of the study infants. Some studies have shown that disease is only found to have a significant effect on growth in New Guinea population from the seventh month[24] or as soon as supplementary food is introduced.[25] Many previous studies have shown that inadequate energy intake is the most likely cause of infants' poor growth.[26] This supplementary food might provide insufficient energy density and micronutrient intake.[27,28] The frequency, quantity, and type of supplementary feeding are strongly associated with stunting.[29] A study in Indonesia suggested that part of growth faltering, especially in the second half of the first year, was due to inadequate food intake.[30] The falling of growth beginning from about 4 to 6 months of age in the study highlights the need for increased attention to intervention and adequate supplemental feeding.

In conclusion, this study contributes to the understanding of the complex pattern growth of LBW at term infants in the first year of life. Differences with respect to NBW infants in weight and length gain by LBW at term infants in the first year of age were not statistically significant.

Weight and length growth by LBW at term infants is greater in the first 4 months of age and can still catch-up to normal at some point during the first year of life. At 1 year of age, the nutritional status (z-scores) of LBW infants tended to improve compared with their status at birth. However, these infants will not achieve the same weight or length curves at 12 months of age as the NCHS standard recommended for infants of the same gender and age.

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1. 女性生殖器における免疫機構

早川 智，真島洋子，相澤志保子

女性生殖器粘膜における免疫応答は，消化器や呼吸器とは異なった特徴を有する．すなわち，膣や子宮内膜（脱落膜）には月経周期や妊娠，閉経など刻々と変化する内分泌環境の影響を強く受けて，ダイナミックな機能的変化をとげる局所免疫系が存在する．未熟なNK細胞やNKT細胞，マクロファージ，樹状細胞などからなる女性生殖器粘膜免疫系は宿主を感染症から防御すると同時に，非自己である精子，胎児胎盤を許容して妊娠を成立させる．妊娠現象とその破綻，また，HIV感染を含む性行為感染症（sexually transmitted disease：STD）や垂直感染の理解と制御には女性生殖器粘膜における免疫応答の理解が必須である．

はじめに

個体の寿命が有限であることの必然として，遺伝子はその乗り物を次々に変えてゆく必要がある．バクテリアのように無性生殖で同一のコピーを増やすことがもっとも手っ取り早く，コストがかからないが，プラスミドなどによる遺伝子の交換には自ら限界がある．雌雄両性による有性生殖の本質は世代ごとの遺伝子のシャッフリングにより，環境の変化，特にあらたな病原微生物の出現や変異に対する宿主の多様性を確保することにあると考えられている．それには異なった遺伝的背景を有する個体との間で情報の交換が必須であり，さらに子宮内で胎児胎盤を育てる真胎生動物では胎児胎盤を許容するため特異免疫系と折り合いをつけねばならない．

1953年，Sir Peter Medawarが免疫学的異物である胎児胎盤がなぜ，拒絶されないのかという生殖免疫学の中心的命題を提起して半世紀が過ぎた現在でも，妊娠現象は謎に満ちている．彼が想定した四つの仮説，① 母体の免疫応答は妊娠中低下する．② 胎児胎盤は免疫学的に未熟である．③ 子宮腔内は免疫学的に特異な場所であり，免疫応答が生じない．④ 胎児循環と母体循環は胎盤によって完全に隔離されている．①は，現在ではいずれも否定され，1）胎盤におけるextravillous trophoblastは単型のHLA-Gを発現し細胞傷害性T細胞（cytotoxic T lymphocye：CTLを誘導できないと同時にNK細胞のネガティブシグナルとなる．2）脱落膜局所のリンパ球は活性化し種々のサイトカインを分泌するがこれは胎児胎盤の成長を促進する（immunotrophism）[2]．3）脱落膜局所に制御

[キーワード&略語]
脱落膜，生殖内分泌，有性生殖，γδT細胞，胸腺外T細胞，Th1/Th2パラダイム

dNK細胞：decidual Natural Killer細胞（脱落膜NK細胞）
STD(STI)：sexually transmitted disease (sexually transmutted infection)（性行為感染症）

Mucosal immune system of female reproductive tract
Satoshi Hayakawa/Hiroko Majima/Shihoko Komine-Aizawa：Division of Microbiology and Infectious Diseases, Department of Pathology and Microbiology, School of Medicine, Nihon University（日本大学医学部病態病理学系微生物学分野）

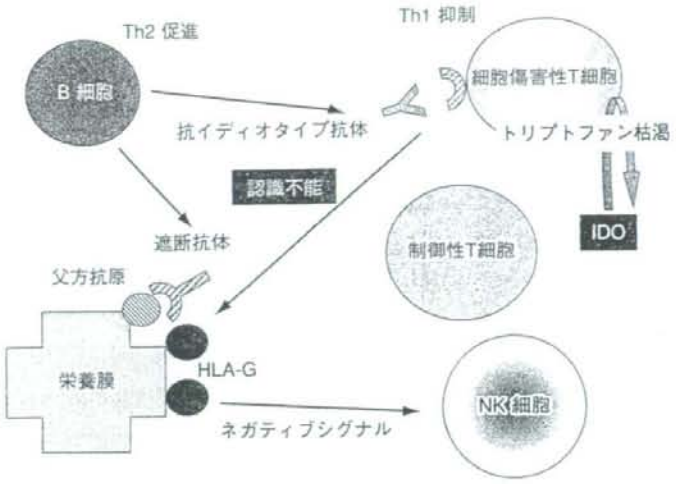

図1　脱落膜における免疫応答
母体との接点にある栄養膜細胞は単型のHLA-Gを発現し，細胞傷害性T細胞の認識を免れると同時にNK細胞に負のシグナルを送る．妊娠中はTh2優位の免疫環境となり，細胞傷害性T細胞の機能は抑制される．胎盤が産生するIDOは局所のトリプトファンを代謝して細胞傷害性T細胞の機能は抑制する一方，制御性T細胞の機能を活性化する

性T細胞[3]やTr1細胞など抑制性の細胞が存在する．4）妊娠中はTh2優位の免疫学的環境にある．5）IDO（indoleamine-2, 3-dioxygenase）による局所のトリプトファン欠乏がCTLを抑制する[4]．などの機構が関与すると考えられている（図1）[5]．

1 子宮内膜・脱落膜の粘膜免疫

特異免疫系の起源は有顎脊椎動物の消化器粘膜にある．[6] 消化器粘膜における免疫応答系は進化のうえで，その祖形に最も近いものであり，呼吸器や生殖器における粘膜免疫や皮膚の免疫はこれから派生したものと考えられる．粘膜免疫の研究は消化管を中心に進められてきたが，女性生殖器における粘膜免疫応答は① 月経周期など内分泌因子による調節を受ける．② 異物である精子・胎児抗原の認識と受容を行う．という2点で他の局所免疫系とは異なった側面がある．臨床的には① STDの侵入門戸となる．② 妊娠の生理と病理に直接関与する．③ 婦人科腫瘍の発生と進展に関与する．といった点が重要である．

腟内には細菌叢が存在するが，子宮腔内や卵管内は無菌である．女性生殖器は種（厳密には自己の遺伝子）を保存するために，胎児の生存には危険を及ぼすことなく，病原体から自己を防御する免疫機構を進化させた．胎児に対し，拒絶的に働く個体は子孫を残せない一方，無制限に異物を受け入れる個体は感染症に対する抵抗性を維持できないからである．ここに，胎児の生存を守り，かつ潜在する病原体から保護していくという女性生殖器独自の免疫機構の存在理由がある（図2）．

2 上皮細胞

内性器粘膜の構成細胞は上皮細胞，マクロファージ，樹状細胞（DC），好中球，NK細胞，T細胞，B細胞，NKT細胞などである．上皮細胞はその最前線に位置し，連続的なバリアーとして微生物の侵入を防止する．腟粘膜，子宮内膜とも性ホルモンの直接的な影響を強く受ける．腟から子宮腟部は扁平上皮に覆われ，エストロゲンによってグリコーゲンを産生する．角化して剥離した上皮細胞中のグリコーゲンは腟内のデーデルライン桿菌によって分解されて乳酸となり，腟内を酸性環境に保つことで，雑菌に増殖を抑制する．（腟の自浄作用）

先に述べたように子宮腔内は無菌であるが，性周期を有する女性では月経により約28日毎に剥離脱落と再生を繰り返す．子宮腔と腟内は頸管粘液で遮断され，細菌の侵入を防ぐが原則的に精子の侵入は許す．

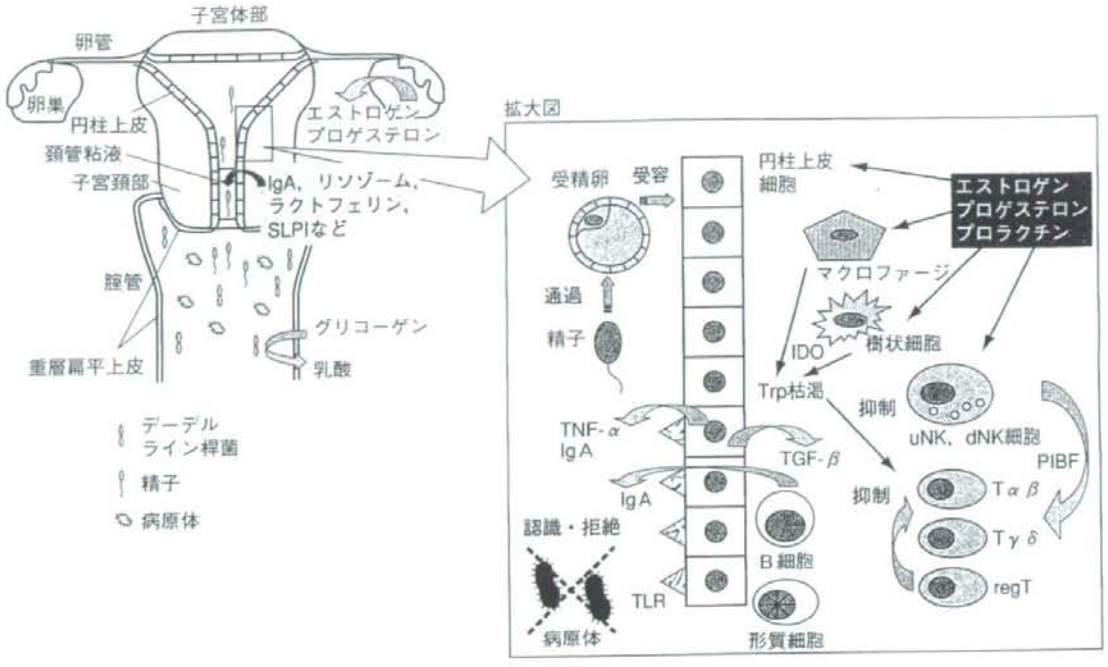

図2　女性生殖器の粘膜免疫
腟上皮の産生するグリコーゲンはデーデルライン桿菌によって乳酸に代謝され，腟内を強酸性に保つ．子宮頸管は粘液栓により，腟腔と隔てられ，頸管内に分泌されるIgA,ラクトフェリンなどが細菌や真菌，原虫，ウイルスの進入を抑制する一方，精子の進入を許す．半非自己（semi allograft）である胎児胎盤は子宮内膜が性ステロイドによって変化した脱落膜に着床し，拒絶されることなく妊娠期間（ヒトでは280日間）を過ごす

頸管腺によって産生される粘液の量や性情はエストロゲンによって調節される．常在菌が存在する腟や子宮腟部は重層扁平上皮細胞に覆われ，機械的損傷に抵抗性があるが，子宮頸管や内膜，卵管の円柱上皮は一層であるため，物理的損傷や感染には脆弱である．しかし，妊卵を許容するためには欠くことのできない構造である．実際，子宮腔内の慢性炎症では扁平上皮化成を見ることがあるが，このような場合には着床不全を伴う．近年，上皮細胞が単なる物理的バリアーとしての役割だけでなく，感染制御に積極的にかかわること，これに性ホルモンが密接に関与することが明らかになった．獲得免疫系は，特異的に活性化して病原体に対する反応性を発揮するまでに一定の時間を要するため，病原体の侵入初期には非特異的な防御機構が作用する．その1つは，上皮細胞による殺菌性の可溶性物質産生である．重要なものにデフェンシンやSLPI（secretory leukocyte protease inhibitor），LF（lysozyme and lactoferrin），tracheal anti-microbial peptideなどがある．実際臨床的に，腟部頸管粘液中のSLPIの濃度は閉経前の女性の方において，閉経後よりも有意に高いこと，月経周期によって変動することが報告されている．妊娠中や羊水中にはさらに増加しており，その分泌はプロゲステロンに依存する．子宮内腔の上皮細胞は構造的，機能的に極性をもった配置をとっており，内腔にTNF-αやIgAを分泌する一方，基底膜下にTGF-βを分泌する．その分子的基盤として，Wiraらは培養上皮細胞を用いてTER（transepithelial resistance）という概念を提唱し，これがエストロゲンによって濃度依存性に調節されていることを明らかにした．上皮細胞は間質細胞とも，直接接触あるいはケモカインやEGF（epidermal growth factor），VEGF（vascular endothelial growth factor）など増殖因子を介した密接な機能的関連を有してしており，これもエストロゲンの支配を受ける．

頸管上皮細胞，子宮内膜上皮細胞はTLRを発現し，微生物の侵入に対応するが，その発現もエストロゲン

の影響を受ける．すなわち，生殖年齢にある女性では微生物侵入の機会も高いためTLRの発現は高いレベルに維持されるが，閉経後はエストロゲンが低下すると同時にTLR発現も低下する．TLRについても原則的に無菌の上部生殖器とフロラのある下部生殖器を分けて考える必要があろう．

3 マクロファージと樹状細胞

単球・マクロファージは病原体や死細胞を認識して貪食する．さらに獲得免疫系に対して抗原提示を行うことにより，防御反応連鎖の第一線を担う．異物認識とその処理にかかわる機能は性ステロイドホルモンを含む可溶性分子の調節を受ける．解剖学的にはマクロファージはヒトの女性生殖器に広く分布し，組織白血球のおよそ10％を占めるが，特に内膜間質と筋層の結合組織の中に多く存在する．月経前の子宮間質に選択的に集まり，黄体消退に伴って他の内膜組織とともに剥離脱落する．Wiraらは子宮内膜マクロファージがER，PRを介して，性ステロイドホルモンの直接的調節を受ける可能性を報告したが，Ariciらは内膜間質細胞をエストラジオール処理することで，MCP-1発現が減少することから，これによるマクロファージの移動抑制が重要ではないかとしている．さらに，ヒトの内膜組織のマイクロアレイ解析により，他のケモカイン，MCP-3，FKN，MIP-1 βなどは月経前に増加することが報告されている．

粘膜免疫からは若干外れるが，マクロファージは卵巣機能においても重要な因子となっている．子宮のマクロファージ同様，卵巣マクロファージの分布は月経周期による変化を示す．マクロファージは血管周囲の結合組織と，排卵前後の黄体の被膜に数多く存在する．排卵は生理的な炎症反応として説明できるが，その調節因子として卵巣のマクロファージはTNF-α，IFN-γ，IL-1，IL-6，IL-10，IL-12などのサイトカインを介し，また貪食作用による黄体融解や細胞外基質の処理を行う．その過程でマクロファージ由来のMMPによる，卵胞の発生と閉鎖の調節が重要な役割を果たす．

一般に自然免疫では，エストロゲンはERαを介してマクロファージの機能を抑制すると考えられている．LambertらによるERαのノックアウトマウス（KO）の実験では，LPSと*Mycobacterium avium*に感作させた腹腔マクロファージを*in vitro*で刺激したとき，ERα −/− 動物では野生型の腹膜マクロファージよりも強くTNF-αを分泌するという．一方では，エストロゲンはB-1細胞や胸腺外T細胞など自然免疫系細胞の活性化を誘導することから，これのみで自己免疫性疾患発生率における性差や治療抵抗性を説明することは難しい．自己免疫疾患と性ホルモンについては興味ある問題であるが，本総説の趣旨から外れるので成書や総説を参考にしていただきたい[7) 8)]．

マクロファージと類縁の細胞に樹状細胞がある．樹状細胞とは樹枝状の突起を伸展させていることを形態的な特徴とし，全身の他の臓器同様，子宮内膜・脱落膜にも一定数が存在する．クラスⅡのMHCを恒常的に発現し，貪食した異物抗原をT細胞に提示することをその主要な機能とする．しかし，実際に抗原特異的な適応免疫応答が誘導されるためには，樹状細胞上にT細胞の活性化に必要とされる種々の補助刺激分子を発現し，サイトカインを産生する必要がある．脱落膜の樹状細胞はDC-SIGNを発現し，これが抑制性の免疫応答に必須であること[9)]，プロゲステロンが脱落膜におけるミエロイド型の樹状細胞の分化に必須であることが報告されている[10)]．宮崎らは脱落膜の樹状細胞がCD80とCD83，CD86を構成的に発現しさらにミエロイド型の表現型であるCD11c$^+$CD123$^-$であること，この細胞がTh2型の免疫応答を誘導することを報告した[11)]．マクロファージ，樹状細胞はトリプトファン代謝の律速酵素で，T細胞の調節因子であるIDOを発現することにより，局所の免疫応答を負に調節する．われわれは，本来細胞性免疫応答の活性化因子であるIFN-γが高濃度のプロラクチン（PRL）存在下ではIDO産生発現を誘導することを明らかにした[12)]．PRLは下垂体前葉より分泌されるが，脱落膜間質細胞でも産生されることから局所の免疫調節機構に関与している可能性がある．

4 NK細胞

子宮内膜と腸管粘膜にはアズル顆粒陽性でCD16$^-$CD56$^+$CD3$^-$の未熟なNK細胞が多数存在する．1920年Weilは腸管粘膜と，脱落膜に存在する大顆粒リンパ球（*Les cellules granuleuses des musqueses in intestinale et uterine*）として報告した[13)]．NK細胞は全身に広く分布するが，末梢血のNK細胞の大部分

が$CD16^+CD56^{dim}$の表現系を示すのに対し，脱落膜NK細胞は，$CD16^-CD56^{bright}$である．$CD56^{bright}$細胞は胎生期初期に現れることや，骨髄移植後早期に出現することからきわめて未熟なNK細胞と考えられている．しかし，近年のマイクロアレイ解析から，子宮内膜NK細胞（uNK細胞）は血中の$CD16^-CD56^+$NK細胞とは異なった独自の細胞集団らしいことが明らかになってきた．この細胞群は妊娠により著しく増加し，脱落膜NK細胞（dNK細胞）として妊娠初期には脱落膜免疫細胞の80％を占めるに至る．現時点ではdNK細胞は血中のNK細胞に由来して，脱落膜という特殊な環境で分化したのか，脱落膜局所で複製しているかは不明であるが，その増殖や分化に性ステロイドが関与していることは間違いない．しかし，リアルタイムRT-PCRでER，PRを欠くとする成績もあり[14]，間質細胞や上皮細胞による間接的な支配を受けている可能性がある．一方，Szekeres-BarthoらはCD56uNK細胞（dNK細胞）がプロゲステロンによって誘導される免疫調節物質（PIBF）を産生し，これがB細胞の遮断抗体産生やTh2優位の免疫応答を誘導することから妊娠維持に必須であるという仮説を提唱している[15][16]．

5 脱落膜T細胞

免疫染色あるいはフローサイトメトリーによって検討すると脱落膜内にはαβもしくはγδのT細胞受容体を有する成熟したT細胞が存在する．しかし，その割合は末梢血に比較して著しく少なく，その性状は長く明らかではなかったが，1994～1995年われわれとLundqvistらのグループが相次いで胸腺を経ないで分化する胸腺外T細胞がその多くを占めることを明らかにした[17][18]．T細胞を欠損したヌードマウスやRAG-1欠損マウスが妊娠可能であることから脱落膜のT細胞が妊娠維持に必須である可能性は低いがT細胞受容体を介した特異的認識が病的状態に関与する可能性がある．斎藤らは脱落膜T細胞ではT細胞受容体（TCR）／CD3複合体がほとんどすべての細胞で低下していること[19]，しかしながらCD69，HLA-DR，IL-2Rβ鎖などの活性化抗原を発現していることを明らかにした[20]．その意義として彼らは脱落膜において胎児胎盤抗原やサイトカインによって活性化されたT細胞に同時に寛容が誘導されたためと説明している．

MHC-TCRを介したactivationやanergyでは特定のエピトープを認識するクローンのみがTCRの発現低下や活性化マーカーの表出を行うのに対して脱落膜では全T細胞がこのような変化を示すことから抗原非特異的な刺激の存在や未熟な胸腺外T細胞としての性状である可能性がある．われわれはさらに脱落膜T細胞が$CD161^+$に加えて，NKT細胞特異的なTCR Vα24を使用することを明らかにした[21]．胸腺外T細胞，NKT細胞はともにエストロゲン受容体を介してその機能を調節されるが[22][23]，抗原非特異的な感染防御や過剰な炎症反応の調節に関与するのみならず，胎児胎盤認識のうえで何らかの生理的な役割を果たしていると考えられる．正常妊娠脱落膜T細胞，NK細胞はともに通常の培養条件では自己絨毛細胞や絨毛癌細胞株に細胞傷害性を有さないがIL-2やIL-12の刺激によって傷害性を獲得する[24]．興味深いことに妊娠中に胎盤が産生するG-CSFはIL-2やIL-12によって活性化した脱落膜リンパ球の絨毛細胞傷害性を強く抑制する[25][26]．妊娠時のG-CSFは一義的には局所の免疫抑制因子として作用し，母体の白血球増加は二次的な現象のようである．

6 γδT細胞

子宮内膜には他の粘膜や表皮と同じくγδT細胞が存在する．γδT細胞の生理的機能は不明な点が多いが，多様性のない自己MHC類似抗原（CD1やTLaなど）や熱ショックタンパク質HSP60を認識し，初期T細胞防御反応として病原体や障害細胞の除去に働く一方，過剰な免疫反応を抑制し組織破壊を防御すると考えられている．Mincheva-Nilssonらは免疫電顕法によって脱落膜にはCD56陽性のγδ陽性T細胞が多数存在しT細胞の活性化マーカーであるCD45ROを発現していることを明らかにした．マウスでは脱落膜γδ細胞はリンパ球混合培養に対して抑制的に作用するが[27]，われわれはこれら可移植腫瘍初期病変に浸潤するγδT細胞と同一の細胞集団であることを明らかにした[28][29]．さらにわれわれは，脱落膜では腫瘍内リンパ球やアレルギー患者鼻粘膜[30]と同一の抑制性$V\gamma I\delta 1$細胞が多く存在しIL-10やIL-13などTh2サイトカインやTGF-βを産生することを報告した[31]．先に述べたようにマウス脱落膜に存在するγδT細胞はTGF-β2を産生しアロの免疫応答を抑制することが知られて

いる[32]～[34]．

近年，マウス脱落膜γδT細胞は均一の細胞集団ではないことが明らかになってきた．Clarkらは習慣性流産モデルマウスとして有名なDBA/2VXBA/Jの系において妊娠6.5日asialo GM1陽性Vγ1.1δ6.3T細胞がIFN-γを産生して着床を阻害するのに対して，妊娠8.5日にみられる同一のT細胞受容体を使用する細胞はIL-10やTGF-β2を産生し妊娠維持に働くとしている[35]．彼らによるとNKマーカーであるasialo GM1の有無がサイトカインの産生パターンと密接に関係しているという．すなわちasialo GM1陽性のγδNKT細胞は胎児胎盤に傷害性に作用しasialo GM1陰性の古典的γδT細胞はTh3やTr1の表現型を有し妊娠維持に働くらしい．また，抑制性のγδT細胞からのシグナル伝達経路には新たに発見された抑制性分子OX-2が関与するという．彼らは局所のTh1/Th2Th3比がそのまま妊娠予後に結びつくわけではないがLPSやストレスによる流産感受性に強く相関するとしている．γδT細胞の分化も，性ステロイドの支配を受けることが知られており，PIBFの関与が強く示唆されている．

7 Th1/Th2パラダイムとその限界

抗原特異的な免疫応答において中心的な役割を担うCD4陽性のヘルパーT（Th）細胞はB細胞による抗体産生や細胞傷害T細胞の機能を調節する．1990年代には，Th細胞が産生サイトカインによってTh1Th2の2種類に大別されること，そして2つのバランスの変化が自己免疫疾患やアレルギーなどの病態に密接に関係していることが明らかになった．Th1優位の免疫応答が生じるマラリアやリステリア感染では流早産がみられることから，妊娠の維持にはTh2優位が必須と考えられている．脱落膜局所におけるTh2の優位を示唆する所見として，Piccinniらはヒト正常妊娠脱落膜より樹立したT細胞クローンの多くがTh2サイトカインを産生するが流産患者では低下することを報告し[36]，斎藤らは子宮内膜T細胞におけるTh1/Th2細胞比を解析し妊娠初期にTh2が優位であることを明らかにした[37]．脱落膜局所にTh2優位の環境を誘導する因子としてマクロファージが産生するIL-10に加えてPGE2が重要と考えられている[38]．また胎盤で産生されるプロゲステロンやhCGが直接的あるいは間接的にTh2型の免疫応答を誘導すると考えられるが，これに加えてわれわれはTh2特異的なケモカインであるTARCの関与を明らかにした[39]．さらに長時間同一抗原に暴露するとTh1細胞が選択的にアポトーシスに陥ることが知られており[40]，妊娠中は胎児胎盤抗原の存在自体がTh2を誘導している可能性がある．妊婦末梢血でもTh2優位となっている知見としてFCMにより細胞内サイトカインを定量すると妊娠中後期ではTh1/Th2比がTh2優位に傾くこと[41]，夫リンパ球で妊婦末梢血を刺激すると選択的にTh2サイトカインが産生されること[42]，などが報告されている．

習慣流産において，全身的にTh2が抑制されTh1優位となっていることを示唆する所見としては，末梢血T細胞をPHAなどの非特異的なmitogen[43][44]や絨毛癌細胞株由来抗原[45]，当該妊娠胎盤絨毛細胞[46]で刺激したときに習慣流産患者では健常妊婦に比較してIL-2やIFN-γなどtype-1サイトカインの産生が増強しtype-2サイトカインであるIL-10の産生が抑制されること，FCMで細胞質内にTh1サイトカインが染色されるCD3細胞が有意に増加していること[47]が報告されている．われわれは習慣流産患者に対し夫リンパ球による免疫療法を施行するとTh1/Th2比が低下し，これが妊娠予後とも相関することを報告した[48]．しかしながら，Th1サイトカインであるIFN-γ受容体をノックアウトしたマウスは不妊であり[49]，着床初期にはTh1型の炎症反応による血管構築の誘導が必須であると考えられる．また，われわれが行ったマウスにおけるサイトカイン活性化リンパ球移入実験では，Th1あるいはTh2いずれにも過剰活性化した動物では胎児胎盤の吸収と妊娠中毒症様の症状が認められた[50]．妊娠はTh2善玉Th1悪玉という簡単なスキームでは必ずしも十分な説明はできない[51]．

8 抑制性免疫応答の内分泌支配

真に免疫応答に対する負の制御を行うには，抗原特異的に活性化してTh1，Th2いずれもの応答も抑制する必要がある．この機能を担うのが坂口らにより発見された制御性T細胞である．$CD4^+CD25^+$の表現型を有するこの細胞は，免疫自己寛容を維持し，自己免疫疾患の発症阻止に関与するのみならず，感染症においては過剰な応答による組織破壊から宿主を防御し，また妊娠の維持に関与する．健常人では末梢血の5～

10％を占めるが，基底側脱落膜T細胞では70～80％に達する[52]．実際機能的にも，マウスならびにヒトの系で，着床と妊娠維持に制御性T細胞が必須であることがAluvihareらおよび[53]佐々木らにより報告された[54) 55]．制御性T細胞の誘導ならびに調節の分子機構は，癌の免疫療法や臓器移植の制御にもつながるため近年，注目を集めている領域であるが，近年，複数の施設よりエストロゲンがFOX-P3の発現をup-regulationすることにより，制御性T細胞の活性を調節することが明らかにされた[56)～58]．性ホルモン以外にも，胎盤，脱落膜で産生される複数のホルモンや神経伝達物質が局所の免疫応答を調節している可能性がある．われわれはβエンドルフィンがμ受容体非依存的にNK細胞，NKT細胞のサイトカイン産生を調節することを明らかにした．またβエンドルフィンはTLRを介したIFN-αの産生も負に制御することが明らかになった．胎盤は中枢神経，副腎髄質に次ぐエンドルフィンの産生臓器であるが，産生されるのはμ受容体に結合活性のないN-アセチルエンドルフィンであり，その生理的機能は謎であったが，われわれの研究より局所の免疫応答にかかわる可能性が示唆される．

おわりに

女性生殖器における粘膜免疫は，内分泌支配を受けていることが他の臓器と大きく異なる．その解析により，妊娠機構の解明や妊娠合併症の予防，性行為感染症を含む感染症の解析と制御に新たな道が開ける可能性がある．

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＜筆頭著者プロフィール＞
早川　智：現職 日本大学医学部病態病理学系微生物学分野教授．1983年日本大学医学部卒業，'87年同大学院医学研究科修了，'85～86年City of Hope研究所生殖遺伝学部門ポスドク（大野乾研究室），'91年日本大学医学部助手（産婦人科学），'98年国立感染症研究所エイズ研究センター協力研究員（併任），2004年日本大学医学部助教授（先端医学講座・感染制御科学）'06年ラオス国立大学客員教授（感染症学），聖母大学看護学部客員教授（産婦人科学），'07年より現職．専門 生殖免疫学，産婦人科学，進化医学，医学史

ORIGINAL ARTICLE

Results of Immunotherapy for Patients with Unexplained Primary Recurrent Abortions – Prospective Non-Randomized Cohort Study

Taro Nonaka, Koichi Takakuwa, Izumi Ooki, Mami Akashi, Tomokazu Yokoo, Akira Kikuchi, Kenichi Tanaka

Department of Obstetrics and Gynecology, Niigata University School of Medicine, Asahimachi-dori, Niigata, Japan

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Correspondence
Koichi Takakuwa, Department of Obstetrics and Gynecology, Niigata University School of Medicine, 1-757, Asahimachi-dori, Niigata, 951-8510, Japan.
E-mail: obgy@med.niigata-u.ac.jp

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Problem
The present study was conducted to examine the efficacy of immunotherapy for unexplained primary recurrent aborters using paternal lymphocytes.

Method of study
Two hundred and twenty-eight recurrent aborters were prospectively followed up regarding immunotherapy. Of the 228 patients, 165 underwent immunotherapy using freshly prepared paternal lymphocytes and pregnancy outcome was analyzed. No mixed lymphocyte culture reaction-blocking antibodies (MLR-BAbs) were observed in these patients prior to vaccinations. Pregnancy outcome was also analyzed in such as those patients positive for MLR-BAbs and who did not undergo immunotherapy, and in patients negative for MLR-BAbs and who had become pregnant without immunotherapy.

Results
Of the 140 newly pregnant patients after immunotherapy, the pregnancy continued successfully in 110 (78.6%), and the pregnancy continued successfully in 24 of 32 patients (75.0%) who were positive for MLR-BAbs. The success rate of pregnancy was 30.0% in 18 non-immunized patients. Thus, the success rate was significantly higher among patients with immunotherapy and patients positive for MLR-BAbs than in non-immunized patients, negative for MLR-BAbs.

Conclusion
Immunotherapy using paternal lymphocytes is considered to be effective for unexplained primary recurrent aborters negative for MLR-BAbs.

Introduction

Although the etiology of recurrent spontaneous abortion, defined as three or more consecutive early pregnancy losses, is often unclear, several investigators have reported the occurrence of immunologically explainable recurrent spontaneous abortions. Immunotherapy for these patients using the husband's or a third party's leukocytes has been reported for the past quarter of a century.[1–7] The efficacy of this modality, however, is controversial, even among studies with randomized controlled trial.[8–11] This is mainly because of the selection criteria that were adopted, or of the procedure of the immunotherapy. In this study, we show the results of immunotherapy for unexplained primary recurrent aborters using freshly prepared lymphocytes from the husband and attempt to examine the efficacy of the therapy, especially in patients negative for blocking antibodies evaluated by a mixed lymphocyte culture reaction between spouses (MLR-BAbs).

Materials and methods

Patients

A total of 228 primary recurrent aborters, presenting between January 1983 and December 2005, took part in the study. All had provided informed consent. Each participant cohabiting with a single partner had experienced three or more consecutive, confirmed first trimester (i.e. before 14 weeks of gestation) spontaneous abortions. All had experienced no other pregnancy, and so were diagnosed as primary recurrent aborters. None of the participants had indication for presence of any genetic impairment, mullerian anomaly, hormonal deficiency, infectious disease, metabolic disorder, or autoimmune abnomalities, such as antiphospholipid antibodies or lupus anticoagulant disorder, in the course of our systemic work-up.

The patients were initially divided into two groups according to the presence or absence of mixed lymphocyte culture reaction – blocking antibodies (MLR-BAbs) in the sera collected at the time of or after the last abortion. Those with MLR-BAbs were excluded from the immunotherapy, and the natural course of their pregnancy was observed. Sufficient information concerning immunotherapy for recurrent abortion using paternal lymphocytes was given to the patients who were negative for MLR-BAbs, and the immunotherapy was applied only to those patients who requested it. With respect to the patients who did not desire the immune-therapy, the natural course of their pregnancy was observed. If the pregnancy resulted in repeated abortion, the immunotherapy was given at the patient's request.

Thus, the patients were ultimately divided into three groups, that is, those who underwent immunotherapy (group I), those to whom immunotherapy was not given on account of the presence of MLR-BAbs (group II), and those who did not receive immunotherapy at their own request despite being negative for MLR-BAbs (group III). All patients were offered the same degree of care during their pregnancy.

The period of following up the patients, especially those who had not got pregnant, was about 5 years. A patient was deemed to have not gotten pregnant, if the patient concerned had not become pregnant for about 5 years. Concerning the patients who enrolled in this study after January, 2002, the outcome of pregnancy was determined in December, 2006.

Vaccinations Using the Husband's Lymphocytes

The vaccination procedure has been described in detail elsewhere.[3,4,12] Lymphocytes from 100 mL of heparinized peripheral blood of the husband concerned of each patient, in the experimental group, irradiated with 30 Greys of X-rays to prevent any graft-versus-host (GVH) reaction, were suspended in approximately 1 mL of normal physiological saline solution. Each such cell suspension was i.d. injected into the corresponding patient in the experimental group I, immediately after its preparation. Once MLR-BAbs appeared in the sera following a series of vaccinations, the patients were allowed to become pregnant. In our earlier study, the MLR-BAbs were examined after each vaccination.[3] In a recent study, however, the patients underwent two vaccinations 1 month apart, and then given a third vaccination if the MLR-BAbs were not still detected.[4] If the MLR-BAbs could not be detected after the third vaccination, the patients were allowed to become pregnant, and an additional vaccination was given early in the pregnancy.

Mixed Lymphocyte Culture Reaction-Blocking Assay

The blocking effect of sera was investigated in a one-way MLR between spouses. Lymphocytes were collected from heparinized blood via Ficoll-Hypaque gradient centrifugation. Mixed culturing of mitomycin C-treated stimulator cells of the husband and responder cells of the patient was performed for 6 days in a microtiter plate in RPMI 1640 medium containing either pooled human AB serum or test serum. The cultured cells were harvested onto a glass fiber filter after 18 hr of pulsing with ^{3}H-thymidine. DNA synthesis was evaluated by liquid scintillation counting, and the blocking effect (BE) was calculated with the formula

$$BE = (1 - \text{mean cpm of culture in tested serum} / \text{mean cpm of culture in AB serum}) \times 100(\%)$$

A 22% or more MLR-blocking effect was determined as significant, and designated a positive reaction for MLR-BAbs, as reported.[3,4,12]

The procedure used for immunization and the method used for testing MLR-BAbs have been

validated, as one of the authors of this paper has been directly engaged in the immunization and MLR-BAbs test throughout this study.

Statistical Analysis

A non-paired *t*-test was used to analyze whether a significant difference exists among the mean age or the mean number of spontaneous abortions in experimental groups I, II, and III. A chi-squared analysis with Yates' correction or Fisher's exact probability test was used to analyze the probability that pregnancy outcome differed among groups I, II, and III.

Results

The patients accrued in this study are shown in Fig. 1. Of 228 patients, 179 (78.5%) were found to be negative for MLR-BAbs with the MLR-blocking assay, using sera collected at the time of or just after their last abortion. Immunotherapy was given to 156 patients who were negative for MLR-BAbs, at their request. The remaining 23 patients had not opted for immunotherapy, and 18 patients experienced 20 pregnancies without immunotherapy (group III) (pregnancy rate: 78.3%). Of these cases, six pregnancies resulted in normal term delivery, and the remaining 14 pregnancies resulted in repeated spontaneous abortion (Success rate; 30.0%). Nine patients in these unsuccessful cases desired immunotherapy at a subsequent stage. Thus, immunotherapy using paternal lymphocytes was given to 165 patients (group I). Of the 49 patients who were positive for MLR-BAbs, 32 have so far experienced further pregnancy later (group II) (pregnancy rate; 65.3%).

The mean patient age and mean number of abortions did not differ significantly among these three groups (Table I).

For 25 of the 32 patients (78.1%) of group II, the pregnancy culminated in delivery. Of these 25, 21 gave birth to mature infants. One light-for-date infant (an infant whose body weight was less than the 10th percentile of the distribution of the general population (neonates) in Japan[13]) was born in the 38th week of gestation with no anomalies, and one infant was born premature in the 31st week of gestation. Three patients had infants with a major anomaly: one of these infants died just before delivery due to severe omphalocele, one was saved by surgery for intestinal atresia, the other was saved by surgery for meconium peritonitis. Pregnancy had resulted in repeated spontaneous abortion in seven cases. Thus, the success rate in this group was 75.0% (24 of 32).

As mentioned above, 165 patients had undergone vaccination with their husbands' lymphocytes. MLR-BAbs were detected after one or two vaccinations in 148 of these, and after the third vaccination in 14 more patients. Thus, the MLR-BAbs were found in 98.2% of vaccinated patients. In the remaining three patients, no MLR-BAbs were detected even after the third vaccination, and an additional vaccination was given early in the pregnancy.

Of 165 patients, 140 experienced new pregnancies (pregnancy rate; 84.8%), and 110 had their pregnancy continue successfully (success rate: 78.6%). All of these 110 patients have already experienced delivery, and 101 delivered normal mature infants in the 36th week of gestation or later. Four light-for-date infants were born at 36 weeks of gestation or later with no anomaly, and four infants were born as premature delivery in the 28th, 32nd, 33rd, and 34th week of gestation. A major fetal anomaly was observed in one infant diagnosed as having Delange syndrome, who had survived after delivery. Pregnancy resulted in repeated spontaneous abortion in the remaining 28 cases, and in ectopic pregnancy in two cases.

The outcome of pregnancy in groups I, II, and III is shown in Table II. The rate of success was significantly higher in group I and II than group III (78.6% *versus* 30.0%, $P < 0.000001$, 75.0% *versus* 30.0%, $P < 0.001$, respectively).

The outcome of pregnancy in group I according to the number of vaccination(s) necessary to test positive for MLR-BAbs is shown in Table III. The rate of successful pregnancy among the patients in whom the MLR-BAbs appeared after one or two vaccination(s) was 76.0% (96 of 125 cases). In the patients in whom the MLR-BAbs showed up after three vaccinations was 91.7% (11 of 12 cases). The rate was 100% in the group of patients in whom MLR-BAbs could not be observed after three vaccinations and an additional vaccination was applied at an early stage of pregnancy (three of three cases). The success rate did not differ significantly among these three groups.

Discussion

The outcome of immunotherapy using the husband's lymphocytes for unexplained primary recurrent