

## SHORT REPORTS

### Etiology of Vitiligo. A New Hypothesis

ABBURI RAMAIAH,<sup>1</sup> NEELU PURI<sup>1</sup> and MANOJ MOJAMDAR<sup>2</sup>

<sup>1</sup>Department of Biochemistry, All India Institute of Medical Sciences and

<sup>2</sup>Institute of Pathology, Safdarjung Hospital, New Delhi, India

**Serum from actively repigmenting human vitiligo subjects had maximum mitogenic effect on the growth of melanocytes in culture, followed by the serum from normal donors, and from untreated vitiligo subjects in that order. Based on these findings, a new hypothesis is suggested for the etiology of vitiligo. Key words: Melanocyte; Growth; Human serum.**

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A. Ramaiah, Department of Biochemistry, A·I·I·M·S New Delhi - 110029, India.

Melanocytes cultured from newborn human foreskins grow faster and can be passaged to 60 or more population doublings, in contrast to those obtained from adult skins which have longer doubling time and can be passaged only 3–4 times (1, 2). Melanocytes derived from uninvolved and perilesional skin of vitiligo subjects manifest a decreased seeding capacity, grow after a long latent period or do not grow at all, and cannot be passaged (1). In contrast, in melanocytes obtained from treated individuals with actively repigmenting vitiligo patches, these defects are completely corrected (3).

Some of the growth defects of the melanocytes obtained from normal skin regions of the vitiligo individuals can be partially corrected *in vitro*, by adding fetal lung fibroblast derived growth factors (3–5). These growth factor(s) not only enhance the growth rate, but also promote the passage capacity of melanocytes obtained from normal donors and uninvolved skin of vitiligo subjects, while withdrawal of growth factor(s) results in the loss of their growth and passage capacities (5).

In the present communication the effects of serum from normal healthy subjects, and patients with vitiligo and actively repigmenting vitiligo, on the growth characteristics of melanocytes cultured from different sources are presented. Based on these results it is suggested that a decreased concentration of melanocyte growth factor(s) in the serum may result in vitiligo.

## MATERIALS AND METHODS

### Materials

Eagle's minimum essential medium, the non-essential amino acids, fetal calf serum and antibiotics were from Gibco, Grand Island, New York. Cholera toxin (CT), 12-*O*-tetradecanoylphorbol 13-acetate (TPA) and trypsin were purchased from Sigma Chemical Company, St. Louis, Missouri, Consolidated Midland Corporation, Katonah, New York, and Difco, USA respectively. Other chemicals used were of the highest grade available.

### Human sera

After obtaining informed consent from the human subjects, we collected blood by vein puncture. The clotted blood was centrifugated and the sera was aspirated and stored at  $-70^{\circ}\text{C}$  until further use. The vitiligo patients were treated with 8-methoxypsoralens and natural sunlight radiation therapy and the serum from actively repigmenting patients was collected at least 24 h after ingestion of psoralens.

### Melanocyte cultures

The adult human melanocytes from normal or uninvolved skin of vitiligo subjects were grown in primary cultures as described earlier (1) in presence of 20% serum from normal, vitiligo, actively repigmenting vitiligo human subjects or fetal calf. Primary cultures rather than pure cultures of melanocytes were used in these studies, since melanocytes from the uninvolved skin of vitiligo subjects could not be passaged without adding fetal lung fibroblast growth factor(s) (1). Even in primary cultures, the growth of melanocytes could be easily followed by *in situ* counting, since adult human melanocytes with their dendrites are easily identifiable under phase contrast microscopy. *In situ* counting of 10 randomly selected microscopic fields ( $0.49\text{ mm}^2$ ), *ad modum* Aubock et al. (6) was done at various intervals in order to measure the melanocyte growth rate. Epidermal cell suspensions from normal human epidermis or from the uninvolved skin of vitiligo subjects were prepared as described earlier (1) and  $0.25 \times 10^6$  epidermal cells from normal human epidermal cell suspension, or  $0.75 \times 10^6$  epidermal cells in the case of epidermal cell suspension from the uninvolved skin of vitiligo subjects, were plated in wells of 24-well Linbro culture plates, as described earlier (1). The growth of melanocytes as described above was followed by *in situ* counts every 48 h.

## RESULTS

The effects of serum at 1–20% from normal donors, vitiligo patients and actively repigmenting vitiligo subjects on the growth of primary cultures of melano-

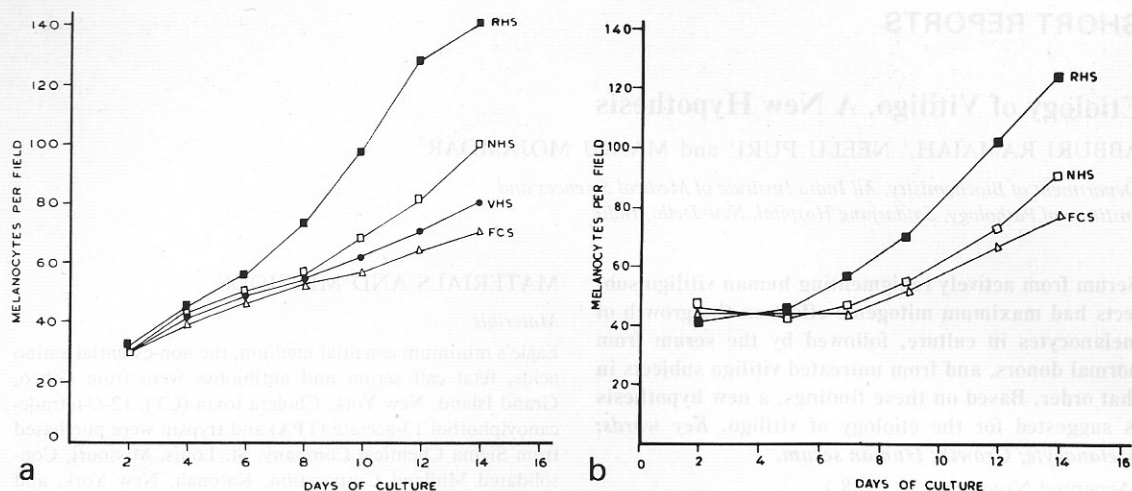


Fig. 1. Typical primary culture growth characteristics of normal donor (a) and uninvolved skin melanocytes of vitiligo subjects (b) in normal human (NHS), repigmenting vitiligo human (RHS), vitiligo human (VHS) and fetal calf (FCS) sera. The concentration of serum was 20% in all cases and was optimum, based on the studies using 1–20% serum. Details of the culture and counting techniques are given in the Ref. 1. The media was changed every 72 h. The numbers given on the Y-axis represent the mean numbers of melano-

cytes from normal donors and uninvolved skin of vitiligo subjects were studied. 20% serum was found to be more effective than at lower concentrations; 20% serum was therefore used throughout. The growth of melanocytes in presence of 20% fetal calf serum was also studied for comparison. The typical growth rates of melanocytes in presence of various sera are presented in Fig. 1a, b. The number of sera tested were 7 each from normal and repigmenting vitiligo subjects and 4 from untreated vitiligo subjects. The effects of these sera were tested on melanocytes cultured from 7 normal donors and 2 vitiligo subjects. The statistical significance of these studies are presented in Table I.

It is clear from these results that serum from normal individuals has mitogenic factors different from those present in fetal calf serum. It was also observed that the serum from repigmenting vitiligo subjects had a significant stimulatory effect on the growth of melanocytes from normal individuals, as compared with the effect of serum from normal (+41.7%  $\pm$  5.1,  $p < 0.001$ ). Similarly, the serum from normal adults had a significant stimulatory effect on the growth of melanocytes from normal donors, as compared with the effect of serum from untreated vitiligo subjects

cytes counted in 10 randomly chosen microscopic fields (0.49 mm<sup>2</sup>). The standard deviation was less than 10%. In situ melanocyte counts were taken until the time every melanocyte could be easily distinguished (2 weeks). After this time, especially in presence of RHS, clustering of melanocytes occurs and counts of melanocytes could become unreliable and therefore counting beyond 2 weeks were not attempted. Similar results were obtained with serum in 7 normal subjects, from 7 repigmenting patients and from 4 vitiligo patients.

(+17.5%  $\pm$  1.1,  $p < 0.001$ ). Similar trends were seen when melanocytes from uninvolved skin of untreated vitiligo subjects were used for these studies (+34.9%  $\pm$  1.9,  $p < 0.05$ ).

These results suggest the presence of mitogenic factors in human serum and that its level is lower in the serum from the untreated vitiligo subjects. This idea is further supported by the following lines of evidence. The melanocytes grow in clusters (Fig. 2a, b), in presence of serum from actively repigmenting vitiligo subjects a feature also seen in melanocytes grown in the presence of optimal levels of fetal lung fibroblast derived growth factors (3–5). Our preliminary observations indicate that 8-methoxypsoralens (25–400 ng/ml) had no effect on melanocytes grown in presence of 20% fetal calf serum, but had a potentiating effect in presence of human serum, suggesting that psoralens have potentiated the effect of factors in human sera on the growth of cells and that such factors are not present in fetal calf serum. The more stimulatory effect of serum from repigmenting treated vitiligo subjects could be either due to an increase in growth factors in this serum, or simply a result of potentiation of its effect by residual psoralens that may be present in serum from treated vitiligo sub-

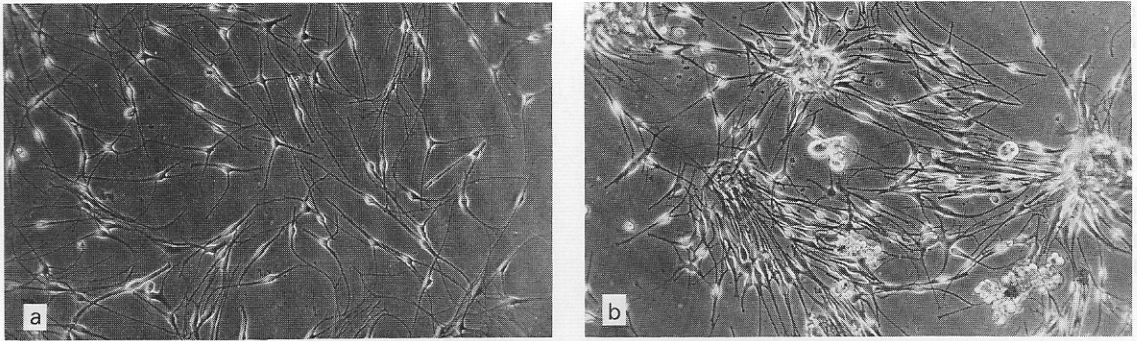


Fig. 2. Melanocytes in primary culture grown in normal human (a) and repigmenting human (b) serum. Note clustered growth in (b). Melanocytes were from the uninvolved skin of a 20-year-old male, vitiligo subject. NHS was from a 20-year-

old male. RHS was from a 20-year-old female vitiligo subject undergoing psoralen + sunlight therapy for 24 months. Similar results were obtained with one other vitiligo subject. Phase contrast,  $\times 92$ , after 32 days of primary culture.

jects. The latter possibility is in our view less likely, because the level of psoralen in the serum of patients 24 h after its intake was zero in 15 cases out of 36 (7), and the effect of serum—even when collected 72 h after the intake of 8-methoxypsoralens, from repigmenting vitiligo subjects—on the growth of melanocytes was same as that of serum obtained 24 h after the intake of psoralens.

## DISCUSSION

Current theories on the etiopathogenesis of vitiligo have several drawbacks and do not seem to be applicable universally to all patients (8). Several workers

have presented a composite hypothesis, combining the three most popular theories—the neural (9) the free radical (10) and the immune mechanism (11). Based on these results presented here, we propose that the normal population densities of melanocytes in the skin may be regulated by growth factors originating both from keratinocytes, fibroblasts (4, 12–14) and from circulation originating from various tissues (15, 16). These growth factors may turn out to be the basic fibroblast growth factor(s) based on the work of Halaban et al. (16) who showed that it has a mitogenic effect on melanocytes in culture. Depigmentation in vitiligo subjects could be due to a reduction of growth factor(s) levels, locally and in circulation, which are

Table I. Effect of different sera on the growth of human melanocytes after 14 days, except in no. 4 which is on the 16th day

Subjects 1–7 are healthy volunteers and nos. 8–9 uninvolved skin of vitiligo patients

Subject no.	Mean number of melanocytes $\pm$ SD in 0.49 mm <sup>2</sup>		
	Normal subjects	Serum from repigmenting vitiligo patients	Untreated vitiligo patients
1	100 $\pm$ 4.9	142 $\pm$ 6.1	84 $\pm$ 5.0
2	100 $\pm$ 4.9	135 $\pm$ 5.6	81 $\pm$ 3.3
3	78 $\pm$ 4.6	110 $\pm$ 7.1	64 $\pm$ 3.7
4	98 $\pm$ 3.4	133 $\pm$ 4.5	N.D.
5	124 $\pm$ 7.3	N.D.	103 $\pm$ 4.8
6	91 $\pm$ 2.6	132 $\pm$ 4.1	N.D.
7	96 $\pm$ 4.1	136 $\pm$ 5.8	N.D.
8	80 $\pm$ 3.0	121 $\pm$ 5.5	N.D.
9	90 $\pm$ 5.0	123 $\pm$ 5.4	N.D.
	103 $\pm$ 4.2	137 $\pm$ 7.2	N.D.

necessary for the normal proliferation and maintenance of the melanocytes.

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#### REFERENCES

1. Puri N, Mojamdar M, Ramaiah A. In vitro growth characteristics of melanocytes from adult normal and vitiligo subjects. *J Invest Dermatol* 1987; 88: 434-438.
2. Eisinger M, Marko O. Selective proliferation of normal human melanocytes in vitro in the presence of phorbol ester and cholera toxin. *Proc Natl Acad Sci USA* 1982; 2018-2022.
3. Puri N, Mojamdar M, Ramaiah A. Defective growth capacities of melanocytes from vitiligo subjects are fully corrected in repigmenting cases and can be partially corrected by fibroblast derived growth factors in vitro. 1st European Pigment Cell Society meeting at Sorrento, Italy. Abstr. (page 47).
4. Mojamdar M, Puri N, Ramaiah A. Effect of mitogenic factors extracted from fetal lung fibroblasts on the in vitro growth of melanocytes obtained from normal and vitiligo subjects. *J Biosciences* 1987; 11: 399-407.
5. Puri N, Mojamdar M, Ramaiah A. Mitogenic factors extracted from fetal lung fibroblast promote the replicative life spans of melanocytes from normal and vitiligo subjects by delaying their terminal differentiation [manuscript communicated].
6. Aubock J, Kofler D, Sifter M, Fritsch P. Application of the tyrosinase assay to normal melanocytes in culture. *Br J Dermatol* 1983; 109: 418-419.
7. Chakrabarti SG, Halder RM, Johnson BA, Minus HR, Pradhan TK, Kenney JA. 8-methoxypsoralen levels in blood of vitiligo patients and in skin, ophthalmic fluids, and ocular tissues of the guinea pig. *J Invest Dermatol* 1986; 87: 276-279.
8. Ortonne JP, Mosher DB, Fitzpatrick TB. Vitiligo and other hypomelanoses of hair and skin. New York: Plenum Medical Book Co., 1983; 243.
9. Lerner AB, Snell RS, Chanco-Turner ML, McGuire JS. Vitiligo and sympathectomy. *Arch Dermatol* 1966; 94: 269-278.
10. Lerner AB. On the etiology of vitiligo and grey hair. *Am J Med* 1971; 51: 141-147.
11. Bor, S, Feiwei M, Chanarin I. Vitiligo and its etiological relationship to organ specific autoimmune disease. *Br J Dermatol* 1969; 81: 83-88.
12. Eisinger M, Marko O, Ogata S-I, Old LJ. Growth regulation of human melanocytes. Mitogenic factors in extracts of melanoma, astrocytoma and fibroblast cell lines. *Science* 1985; 229: 984-986.
13. O'Keefe EJ, Chiu ML, Paynes RE. Stimulation of growth of keratinocytes by basic fibroblast growth factor. *J Invest Dermatol* 1988; 90: 767-769.
14. Halaban R, Langdop R, Birchall N, Cuono C, Baird A, Scott G, Moellman G, McGuire J. Keratinocytes produce basic fibroblast growth factor a natural mitogen for normal human melanocytes. *J Invest Dermatol* 1988; 90: 566(A).
15. Gilchrist BA, Vrabel MA, Flynn E, Szabo G. Selective cultivation of melanocytes from newborn and adult epidermis. *J Invest Dermatol* 1984; 83: 370-376.
16. Halaban R, Ghosh S, Baird A. bFGF is the putative natural growth factor for human melanocytes. *In Vitro* 1987; 23: 47-52.