Behavior of Chick Primordial Germ Cells Injected into the Blood Stream of Quail Embryos

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Summary: The distribution and behavior of chick primordial germ cells (PGC) injected into quail embryos were examined. PGC from chick embryos at stages 13–14 were injected into the blood stream of quail embryos at stages 15–20. After one day, the quail embryos were examined histologically. The chick PGC in the quail embryos could be readily identified by the histochemical PAS technique, whereas quail PGC were never stained by PAS. When the chick PGC were injected into the quail embryos during stages 15–18, they appeared mostly in the gonadal region of the recipient quail embryos. A few PGC were found at extragonadal sites. When the chick PGC were injected into the quail embryos at stages 19–20, in which the PGC of the recipient quail embryos had finished their migration into the gonads, most of the donor chick PGC were found at ectopic sites, in the head, trunk, and limbs. These results indicate that most of the chick PGC, injected at the earlier stages 15–18, migrated to the gonadal anlagen of the recipient, while following later injection (from stage 19), most of the chick PGC migrated to ectopic sites.

Avian primordial germ cells (PGC) first appear in the epiblast at the early stage of development (Eyal-Giladi et al., 1981), then separate from it and temporarily circulate via the blood vascular system. After leaving the blood vessels, they finally migrate into the gonadal anlagen (Swift, 1941; Fujimoto et al., 1976a; Ando & Fujimoto, 1983). In a previous study on the ectopism of chick PGC (Nakamura et al., 1988), we found that some of the PGC (10–20%) deviated from their normal migratory route and colonized exclusively in the head region. Also, in the case of chick embryos lacking gonads, most of the PGC became concentrated in the head region (Nakamura et al., 1991). Based on these results, the present study was undertaken in an attempt to analyze further the ectopic migration of chick PGC. PGC from chick embryos (as the donor) were injected by the intravascular route into quail embryos (as the recipient), and the behavior and distribution pattern of the injected chick PGC in the recipient quails were examined.

Materials and Methods

Injection of chick PGC into the blood circulation of quail embryos

Eggs of White Leghorn chickens were incubated at 38°C for about 2 days to obtain embryos of stages 13–14 (Hamburger & Hamilton, 1951), when PGC are known to be at their peak of circulation via the blood vascular system (Fujimoto et al., 1976b). For the PGC injection, blood samples from chick embryos at these stages were used, since the blood contains PGC. That is to say, circulating blood (2–5 μl) of stage 13–14 chick embryos was taken from the vitelline blood vessels using a micropipet (30–60 μl gauge), and was injected into the blood stream of quail embryos as follows. Japanese quail eggs were incubated at 38°C for 2–3 days to obtain embryos at stages 15–20, which were used as recipients. First, the surface of the shell of the quail egg was swabbed with 70% ethanol, and a round window (about 0.5 cm in diameter) was cut in the shell. Then, through the window, a blood sample containing donor chick PGC as described above was injected into the vitelline blood vessels of the recipient quail embryos at stages 15–20 using the micropipet (Fig. 1). After the injection, the hole in the shell was covered with sellotape and the embryos were subsequently incubated for one more day. The embryos so obtained were dissected free of yolk and fixed with Rossman’s fluid. They were
dehydrated, embedded in paraffin, and sectioned serially at 7 µm for histological examination.

**Histochemical identification of chick PGC (Fig. 2)**

The sections were double-stained with periodic acid-Schiff (PAS) and hematoxylin for light microscopy. Chick PGC were readily identified by this method as large PAS-positive cells (Meyer, 1960; Fujimoto et al., 1976a), whereas quail FGC exhibited no reaction to the PAS technique (Pardanaud et al., 1987).

**Results**

Both chick and quail PGC were characterized morphologically as large spherical cells with large round nuclei (Fig. 2A,C). However, differentiation of chick PGC from quail PGC could be readily made by PAS-staining as described above (Fig. 2B,D).

Of 75 recipient quail embryos receiving chick PGC, 15 grew well for one more day after the injection of chick PGC without bleeding (Table 1). The distribution pattern of the donor chick PGC in these 15 recipient embryos is summarized in Table 2.

**Injection of chick PGC into recipient quail embryos of stages 15–16**

Most of the donor chick PGC were observed in the gonadal region of the recipient quail embryos. Some of the chick PGC had already invaded the epithelium.

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**Fig. 1.** Schematic diagram showing the method for injecting PGC of the chick (donor) into the quail embryo (recipient). A 2–5 µl blood sample taken from chick embryo at stages 13–14 was injected into the vitelline vessels of quail embryos at stages 15–20.

**Fig. 2.** Light-micrographs of hematoxylin and eosin or PAS and hematoxylin-stained sections of the gonadal regions in chick and quail embryos (both at stage 19). Chick (A) and quail (C) PGC (arrowheads) are clearly recognizable as large spherical cells. The chick PGC (B) are stained for PAS-positive glycogen in their cytoplasm, whereas the quail PGC (D) are not. Hind-gut endoderm (En) of the quail embryo is also PAS-positive. A and C: × 500. B and D: × 300.
Injection of chick PGC into quail embryos

Table 1. Recipient quail embryos examined at one day after injection of donor chick PGC.

<table>
<thead>
<tr>
<th>Stage of embryos injected</th>
<th>Recipients</th>
<th>Bleeding</th>
<th>Deaths</th>
<th>Examined</th>
</tr>
</thead>
<tbody>
<tr>
<td>15–16</td>
<td>28</td>
<td>20</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>17</td>
<td>25</td>
<td>18</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>18</td>
<td>9</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>19</td>
<td>7</td>
<td>2</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>20</td>
<td>6</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>75</td>
<td>45</td>
<td>15</td>
<td>15</td>
</tr>
</tbody>
</table>

Table 2. Distribution pattern of donor chick PGC injected into recipient quail embryos.

<table>
<thead>
<tr>
<th>Stage of quail emb. injected</th>
<th>Emb. no.</th>
<th>Total no. of PGC</th>
<th>PGC in gonads</th>
<th>PGC at ectopic sites in head</th>
<th>PGC at ectopic sites in trunk</th>
<th>PGC at ectopic sites in limbs</th>
<th>Ratio (%) of PGC in gonads to total PGC (Mean ratio)</th>
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<tbody>
<tr>
<td>15–16</td>
<td>Q 1</td>
<td>116</td>
<td>98</td>
<td>0</td>
<td>17</td>
<td>1</td>
<td>84.5 (90.6)</td>
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<tr>
<td></td>
<td>Q 2</td>
<td>48</td>
<td>47</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>97.9 (84.7)</td>
</tr>
<tr>
<td></td>
<td>Q 3</td>
<td>28</td>
<td>25</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>89.3</td>
</tr>
<tr>
<td>17</td>
<td>Q 4</td>
<td>39</td>
<td>38</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>97.4</td>
</tr>
<tr>
<td></td>
<td>Q 5</td>
<td>63</td>
<td>47</td>
<td>6</td>
<td>8</td>
<td>2</td>
<td>74.6 (84.7)</td>
</tr>
<tr>
<td></td>
<td>Q 6</td>
<td>73</td>
<td>60</td>
<td>6</td>
<td>3</td>
<td>4</td>
<td>82.2</td>
</tr>
<tr>
<td>18</td>
<td>Q 7</td>
<td>42</td>
<td>16</td>
<td>6</td>
<td>19</td>
<td>1</td>
<td>38.1 (41.4)</td>
</tr>
<tr>
<td></td>
<td>Q 8</td>
<td>39</td>
<td>15</td>
<td>7</td>
<td>10</td>
<td>7</td>
<td>38.5 (41.4)</td>
</tr>
<tr>
<td></td>
<td>Q 9</td>
<td>86</td>
<td>41</td>
<td>17</td>
<td>22</td>
<td>6</td>
<td>47.7 (15.9)</td>
</tr>
<tr>
<td>19</td>
<td>Q10</td>
<td>81</td>
<td>9</td>
<td>26</td>
<td>36</td>
<td>10</td>
<td>11.1</td>
</tr>
<tr>
<td></td>
<td>Q11</td>
<td>125</td>
<td>17</td>
<td>28</td>
<td>64</td>
<td>16</td>
<td>13.6 (15.9)</td>
</tr>
<tr>
<td></td>
<td>Q12</td>
<td>61</td>
<td>14</td>
<td>8</td>
<td>37</td>
<td>2</td>
<td>23.0</td>
</tr>
<tr>
<td>20</td>
<td>Q13</td>
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<td>5.3</td>
</tr>
<tr>
<td></td>
<td>Q14</td>
<td>75</td>
<td>5</td>
<td>25</td>
<td>29</td>
<td>16</td>
<td>6.7 (6.2)</td>
</tr>
<tr>
<td></td>
<td>Q15</td>
<td>45</td>
<td>3</td>
<td>15</td>
<td>21</td>
<td>6</td>
<td>6.7</td>
</tr>
</tbody>
</table>

Observations of the recipient quail embryos were made at one day after injection of chick PGC.

of the prospective genital ridge (Fig. 3). The ratio of donor PGC in the gonads to the total number of donor PGC in the recipient quails was 84.5%, 97.9% and 89.3% in the 3 recipient embryos, Q1, Q2 and Q3, respectively (Table 2). The mean ratio was thus 90.6%, and about 10% of the donor chick PGC were found to be located at extragonadal ectopic sites, in the trunk and limbs.

*Injection of chick PGC into recipient quail embryos of stages 17–18*

Six embryos were examined at one day after injection (Table 1). When the donor chick PGC were injected into the recipient quails at stage 17, most of the donor PGC (about 80%; Table 2) were observed in the recipient gonadal region (developing genital ridge and its vicinity). When the PGC were injected into stage 18 quail embryos, half of the donor PGC were observed in the recipient gonads, while the other half were observed at ectopic sites, in the head, an area close to the neural tube or neural vesicles, the trunk and limbs.

Such ectopic PGC were usually found in the mesenchyme and small vessels.

*Injection of chick PGC into recipient quail embryos of stages 19–20*
In these cases, the number of donor PGC which could be observed in the gonadal region of the recipient quail embryos was fewer than those in the former two cases. In cases where the injection was carried out at stage 20 of the recipient, the donor chick PGC were increased in number up to about 90% of the total, at ectopic sites, in the head surrounding the neural tube, the trunk and limbs (Figs. 4–6). Such ectopic chick PGC were located in the mesenchyme and small vessels of the ectopic sites.

Discussion

In the present study, chick PGC were transferred into quail embryos by intravascular injection in order to examine the behavior and distribution of the donor chick PGC in the recipient quail embryos. Donor chick PGC were taken as 2–5 μl blood samples from the vitelline vessels of stage 13–14 embryos, since the circulating PGC in the vitelline vessels are at their peak in number at these stages (Fujimoto et al., 1976b).

Previously, Reynaud (1969, 1976) carried out transfer of turkey PGC into chick embryos by intravascular injection. However, since both turkey and chick PGC are PAS-positive, it is not so easy to distinguish the donor PGC from the recipient PGC, even though differences in the nucleocytoplasmic ratio of the PGC between the two species of birds are useful for making a morphological differentiation. In the present study, the quail PGC displayed no reaction to the PAS technique (Pardanaud et al., 1987), so that we could readily differentiate the PAS-positive chick PGC from the quail PGC.

In the present experiments, it was uncertain precisely how many PGC were injected initially into the recipient quail embryos, since the chick PGC were injected as blood samples. Nevertheless, useful observations could be made of where the donor PGC were distributed or appeared in the recipients, and of the ratio of ectopic PGC to total or gonadal PGC.

Concerning the mechanism whereby circulating PGC leave the blood vessels and arrive at the gonadal anlagen, it has been speculated that some kind of attractive
factor released from the gonadal anlagen may act on the PGC (Dubois et al., 1976). In the present experiments, the chick PGC injected into the earlier quail embryos (Stages 15–17) were thought to be affected by such an attractant from the recipient gonadal anlagen, although some of them did become distributed by extragonadal sites as discussed below.

When the donor chick PGC were injected into the earlier quail embryos (at stages 15–17), most of the donor PGC (80–90%; Table 2) appeared in the gonadal region of the recipients, while the other PGC (10–20%) were observed at ectopic sites, in the head, trunk and limbs. This pattern of PGC distribution was coincident with that in normal growth chick embryos, as reported by us previously (Nakamura et al., 1988). When the donor PGC injected at later stages of the recipients (stages 19–20), the donor PGC which became included in the gonadal region were decreased in number (under 16% at stages 19–20; Table 2): most of them were found at ectopic sites, in the head, trunk and limbs. These results suggest that the competence of the recipient gonads for retaining donor PGC in the gonad weakened from stage 19 onwards. In fact, most of the chick PGC migrated to ectopic sites of the recipient quail embryos. The present findings also support our previous conclusion (Nakamura et al., 1988) that definitive settlement of PGC in the prospective gonads was from stage 20 in the chick.

The colonization of bird PGC in the head region is thought to be closely related to the formation of the pre-blood vascular plexus in the area at the early stage of development, and a very sluggish blood flow in the capillary network at that site might allow intravascular PGC to extravasate (Van Limbough et al., 1960; Nakamura et al., 1988). Concerning capillary formation in the chick, Meyer (1964) reported that, at stage 18 of the embryo, the splanchnopleuric arteries became greatly reduced in size and number. Evans (1909) indicated that increasing formation of the pre-blood vascular plexus in the head region occurred at the same stage (stage 18) of the chick embryo. These observations support our conclusions outlined above. It is possible that the PGC which deviated from the normal migratory route could have undergone some change in their nature from the normal ones. However, we do not yet have any clear evidence to confirm such an idea.

It is worthy of note that the data obtained in the present study demonstrate the possibility of producing a germline chimera between the chick and quail, and also indicate that, to make a gonadal chimera, the donor PGC must be injected into the recipient bird during the migratory phase of the recipient PGC. The questions of how long the donor chick PGC may survive and to what degree they can differentiate within the recipient quail embryo require further investigation.

Finally, we should emphasize that ectopic distribution of some PGC in birds is a usual phenomenon during the course of germ cell migration, not an occasional phenomenon as is generally supposed.

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References