Demographic explanation of a remarkable enhancement of repopulation hemopoiesis by heterozygous connexin43 stem cells seeded on wildtype connexin43 stroma

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Running title: Heterozygous-connexin enhancement of repopulation hemopoiesis.

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The paper analyses the results of competitive blood-cell repopulation experiments in which Cx43-WT host mice, whose own hemopoietic stem cells were deleted, are grafted with fetal liver hemopoietic cells; 50% of type Gpi-1a/Cx43-WT competing with 50% of Gpi-1b/Cx43-WT or Gpi-1b/Cx43-HZ or Gpi-1b/Cx43-KO. The data are the percentages, %b, of Gpi-1b platelets, granulocytes, red cells, B-cells, T-cells in bleeds from 22 to 186 days after grafting, and percentages of Gpi-1b HPP-CFCs at 255 days after grafting. The results show that, at more than 4 months after grafting, the %b for HZ leads WT and KO by 10% or more, and that KO can even lead WT. The paper proposes a bipolar influence model for blood formation by hemopoietic stem cells (HSCs) to explain this lead and other features of the data. Influence A is a direct one: the combined effect on HSC-niching and HSC-proliferation of connexin43 is superior to that of the knockout allele. Influence B is a demographic one: HZ foundation mice compensate by having more HSCs than WT. The net outcome of influences A and B is that HZ is the winner.

**Key words:** analysis of variance; competitive repopulation; connexin43 wild-type, heterozygotes, and knock-outs; hemopoietic stem cells; standardised cross-validatory residuals
Introduction

Have connexin43 gap junctions on hemopoietic stem cells a determining role in blood formation? On abutting cells the transmembrane proteins, connexins (Cx), of gap junctions form channels which allow the passage of ions, second messengers, and molecules smaller than about 1kDa. The multigene family of connexins is thoroughly reviewed by (1). Cx43, also known as Cx43a1, is the major connexin in hemopoietic tissue but others have been found: Cx37 in bone and in arteriolar epithelium, and Cx45 & Cx31 in marrow stromal cells (2, 3, 4). Cx43 gap junctions are found between certain stromal cells in marrow (4) but it is undecided which other marrow cells express gap junctions. We are uncertain because of the difficulty of identifying with confidence and in the same experiment both the type of blood cell(s) and a gap junction on a hemopoietic cell (5, 6, 7). Three sources indicate that gap junctions on stromal cells couple them to hemopoietic cells. When impermeant lucifer yellow dye (8) is injected into one stromal cell in fetal liver, many adjacent hemopoietic cells become dye-coupled (4). With confocal microscopy the processes of stromal cells can be seen wrapping smaller rounded cells (which resemble primitive hemopoietic cells) and with TEM and freeze-fracture EM (7) we can see gap junctions between stromal and presumptive hemopoietic cells. Hemopoietic colony-formation ceases reversibly in long-term cultures when gap junctions are blocked (9).

In day-to-day blood-formation gap junctions are rare (4). In the neonate which is actively founding its blood-forming system there are more, especially at the epiphysis and metaphysis (active sites of blood-formation). There are even more in irradiated mice or in those recovering their blood-forming system after cytoablation (7). In culture, gap-junctional communication is readily detected by injecting lucifer yellow into stromal cells, whence it spreads to identified hemopoietic cells (4). Comparing different lines of stromal cells, the more they are coupled to neighbouring cells the better they support the growth of long-term hemopoietic repopulating cells (9, 10). When conductivity of gap junctions in long-term cultures is blocked to 2-3% of normal by amphotericin B (2.5-5×10⁻⁹M) clonal blood-formation is blocked and the inhibition is entirely reversed by its removal (9).

What is the function of these marrow gap junctions? A simple-minded hypothesis for the function of hemopoietic Cx43 gap junctions would be that they are another means, besides the growth factors, to start resting HSCs into cycle, acting on developmentally-early cells (9, 11). With Cx43 knockouts (12), fetal liver stem cells of the three Cx43 genotypes can be compared in the live animal (KO mice die at birth). We here use competitive repopulation (13) to ask if there is any difference between the genotypes.

Materials and methods

*Foundation mice*

C57Bl/6J (B6) mice were used in these experiments, either normal glucose phosphate isomerase-1b (Gpi-1b) animals
or congenic Gpi-1a. Foundation animals were bought from Jackson Laboratories (Bar Harbor, Maine). The Gpi-1b foundation mice had the Cx43 knockout allele (5) bred onto a normal B6 background.

*Grafting material*

We extracted the livers from 15-dpc embryos which were then genotyped. Single-cell suspensions were made in 90% fetal calf serum (Labtech) and 10% dried (Molecular sieves, Sigma, M2635) dimethyl sulphoxide (Sigma, D5879) and stored in liquid nitrogen (standard freezing procedures). We then pooled cells of the same genotype in the four combinations: “host” Gpi-1a/Cx43-WT, donor Gpi-1b/Cx43-WT, donor Gpi-1b/Cx43-HZ, donor Gpi-1b/Cx43-KO (14).

*Competitive repopulation*

To compare the three Gpi-1b/Cx43 genotypes, we raced each of them in Gpi-1a/Cx43-WT hosts against “host” Gpi-1a/Cx43-WT cells (13). The hosts’ HSCs had been deleted with 150 mg/kg busulphan (Sigma B02635) but their Cx43-WT stromal cells were intact (14). Each host was grafted with one liver’s worth of “host” cells and one liver’s worth of donor cells: we counted nucleated cells to confirm equal numbers of each type.

*Experimental design*

<table>
<thead>
<tr>
<th>Table 1. Numbers of mice in 21 experimental categories.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment:</td>
</tr>
<tr>
<td>Days after graft:</td>
</tr>
<tr>
<td>dWT:</td>
</tr>
<tr>
<td>dHZ:</td>
</tr>
<tr>
<td>dKO:</td>
</tr>
</tbody>
</table>

<sup>a</sup> 29 days after treatment with FU; <sup>b</sup> HPP-CFC mice; <sup>c</sup> 14 days after treatment with FU.

In five experiments (Table 1) with bleeds between 22 and 186 days after grafting, we measured in each mouse the percentage %<sub>b</sub> of type Gpi-1b cells in platelets, granulocytes, red cells, B-cells, or T-cells (14). These cell-types range from the short-lived (hours for platelets, less than 5 days for granulocytes, 50 days for red cells) to the long-lived (months for B-cells, as much as a year for T-cells). In experiment II, we also measured for mice surviving to 255 days the percentage of type Gpi-1b high-proliferation-potential colony-forming-cells (HPP-CFCs) (15) as the proportion of the colonies that were not of mixed type. Each experiment had three subexperiments corresponding to the three donor genotypes. The symbol dKO will stand, according to context, for both the donor genotype Gpi-1b/Cx43-KO and the subexperiment in which that genotype is raced against “host” Gpi-1a/Cx43-WT; likewise for dHZ and dWT.
To explore more fully the long-term (more than 120 days) consequences of competitive repopulation, the bleeding at 131 days in experiment I was extended to 186 days in experiment II. The other three experiments, with bleeds at different stages under 100 days, were designed to provide a link between the long-term results and previous short-term studies. All the experiments used aliquots from the same four pools of blood (for dWT, dHZ, dKO, and “host” WT), and all aliquots were handled using standard techniques of competitive repopulation experiments (13, 16-18). The host mice in each experiment were randomly assigned to the three subexperiments.

Statistical method

This paper can be read in two ways. Readers may skip statistical detail and commentary and go directly from an appreciation of Figs.1 & 2 to the explanatory modelling of the Discussion. Readers who press on will be able to judge the reliability of the basic data. We have fitted linear models to the data with corresponding analyses of variance (ANOVAs) in order (i) to quantify the relative contributions of different factors to the variation of the observed percentage %b of Gpi-1b cells, (ii) to reveal the satisfactory behaviour of the cross-validatory residuals and the consistency of six estimates of the standard deviation of within-mouse experimental error, and (iii) to use significance tests as pointers to where explanatory modelling may be justifiably attempted.

Table 2 employs a novel format for ANOVA tables that is both succinct and fully informative, using RMS-values (square roots of the usual Mean Squares) which are on the same percentage scale as %b. There are two levels of random variation in the experiments. For comparisons of donor genotypes, the appropriate error term (for F-tests) is the “between mice” one for which mice are treated as the source of randomness (in the second line of the ANOVA tables). For comparisons involving cell-type, mice are treated as “blocks” (in the terminology of Fisherian experimental design (19)): such comparisons are unaffected by any single “error” contributing additively to all the %b-values of a subexperiment, and the appropriate error term is the “within mice” one (in the bottom line of the ANOVA tables).

The so-called “deleted residuals” in (20) are better described as “standardised cross-validatory residuals” (scv-residuals for short). To calculate the scv-residual for a particular %b-value, that value is first omitted from the data, in the spirit of (21). It is then fitted by the formula under study using only the other %b-values, and standardised by the corresponding estimate of the standard deviation of the least-squares residual. Thus the scv-residual is a valid dimensionless measure of both (relative) experimental error in %b and the extent to which the fitted formula can be treated as a true predictor of %b. It can therefore be used as a criterion for excluding an observation of doubtful reliability, and a histogram of all the scv-residuals can reveal such “outliers”. If a hundred scv-residuals from the ANOVA for an experiment form a bell-shaped histogram between roughly -3 and +3 (i.e. plus or minus 3 standard deviations), the fitting formula that delivers them may be taken to be an acceptable description of the data. All our
residuals are of the cross-validatory sort apart from those used either to calculate the residual Root-Mean-Square (RMS) in the ANOVA tables.

**Results**

Fig.1 shows how the means of $\%b$ (averages over replicate mice) vary with donor genotype and cell-type, in both long-term and short-term experiments.

**Figure 1.** Mean percentage, $\%b$, of donor cells in the five compartments (platelets p, granulocytes g, red cells r, B-cells, T-cells) for donor genotypes dWT (+), dHZ (x), dKO (o).

Fig.2 plots the average of the $\%b$-values for the short-lived platelets and granulocytes and shows that the change over time is very different for the three genotypes. For day-255, the means are those of the HPP-CFC $\%b$-values. The unconnected points are either for bleeds made after FU treatment or on day-131. Day-130 is used for the connection so that the falls in $\%b$ to day-255 are “within mouse” comparisons.
**Figure 2.** Changes over time in the means of the average of the platelet and granulocyte $%b$-values for all 15 subexperiments, and of the HPP-CFC $%b$-values at day 255, showing differences between genotypes for both short-term and long-term experiments. The unconnected points are for bleeds made after FU treatment or on day-131.

**Experiment I (131 days)**

The formula fitted in ANOVA I (Table 2) has an additive structure for the $%b$-value of a particular mouse and cell-type:

$$%b = \text{(Genotype mean)} + \text{(Variation dependent on genotype and cell-type)} + \text{(Random variation for the mouse)} + \text{(Random error)}$$

where “(Genotype mean)” is defined to give equal weight to cell-types. Fig.3 gives the histogram of scv-residuals. One mouse has been omitted from this analysis because a preliminary ANOVA gave it two large scv-residuals: 4.7 for red cells and −2.8 for T-cells.
Table 2. Table entries are: Root-Mean-Square (RMS), superscripted Degrees of Freedom, and (if stated) the F-test P-value, for the 3-factor ANOVAs I, IV, V and the 4-factor ANOVAs II, III₂, III₃. The symbol G stands for donor genotype (dWT, dHZ, dKO), M for Mice within Genotypes, C for Cell-types, and D for Days (130, 186 for II; 22, 57 for III₂; 22, 57, 93 for III₃). Interpreting two entries with different error terms: the P-value for G in experiment I is the probability (less than 0.0005) that an F-test statistic with 2 & 17 degrees of freedom exceeds $42^2 / 8.8^2 = 23$; the P-value for C is the probability (also less than 0.0005) that an F with 4 & 68 degrees of freedom exceeds $14^2 / 3.5^2 = 16$.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>I</th>
<th>II</th>
<th>III₃</th>
<th>III₂</th>
<th>IV</th>
<th>V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between mice:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>42²0*a</td>
<td>63²0</td>
<td>16²0.02</td>
<td>6.1²</td>
<td>38²0</td>
<td>95²0</td>
</tr>
<tr>
<td>M in G</td>
<td>8.870</td>
<td>8.2²1.02</td>
<td>6.59</td>
<td>7.8¹¹</td>
<td>8.3²50</td>
<td>6.0²4.001</td>
</tr>
<tr>
<td>Within mice:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>14⁴0</td>
<td>30⁴0</td>
<td>16⁴0</td>
<td>13⁴0</td>
<td>21⁴0</td>
<td>14⁴0</td>
</tr>
<tr>
<td>D</td>
<td>2.0¹</td>
<td>25⁴0</td>
<td>25¹0.003</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C×D</td>
<td>4.4⁴</td>
<td>9.3⁸0</td>
<td>8.5⁴0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G×C</td>
<td>3.5⁸</td>
<td>4.1¹⁸</td>
<td>6.7²0.01</td>
<td>7.3⁸0</td>
<td>6.2⁸0</td>
<td>8.1⁸0</td>
</tr>
<tr>
<td>G×D</td>
<td>4.6²</td>
<td>8.8²0.01</td>
<td>6.0²</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G×C×D</td>
<td>5.5³0.03</td>
<td>3.6¹⁶</td>
<td>5.2³0.02</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M×C in G</td>
<td>3.6³⁴</td>
<td>3.8³⁶</td>
<td>3.2⁴⁴</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M×D in G</td>
<td>5.0²¹0.03</td>
<td>4.0¹⁸</td>
<td>6.6¹¹0</td>
<td></td>
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</tbody>
</table>

*a 0 stands for $P \leq 0.0005$. 
**Figure 3.** Histograms of standardised cross-validatory (scv) residuals in ANOVAs I, II, IV, V.

The differences between the three genotypes are (statistically) highly significant compared with differences between mice within genotypes. Differences between cell-types are also significant compared with the residual $RMS$ of 3.5%, which estimates both experimental error and deviations from the fitted additive formula. The non-significant G×C interaction reflects the parallelism of the three curves in Fig.1(I): the picture can be crudely summarised by saying that, for each donor cell-type, dHZ leads dKO by 10%, which in turn leads dWT by roughly 7%—a feature of the data that will be taken as our first explanatory challenge. (The least-squares estimates of the genotype means in formula [1] and their individual 99% confidence intervals are 46.8(41,52) for dHZ, 36.8(33,41) for dKO, and 30.0(26,34) for dWT.)

*Experiment II (130, 186, 255 days)*

In addition to unconstrained dependence on genotype, cell-type, and day, the analytical technique allows mouse variation to depend additively on cell-type and day. So the fitted formula can now have a richer structure:
\[ \%b = (\text{Genotype mean}) + (\text{Variation dependent on genotype, cell-type, and day}) \]
\[ + (\text{Random variation for the mouse, dependent on cell-type but the same for each day}) \]
\[ + (\text{Random variation for the mouse, dependent on day but the same for each cell-type}) \]
\[ + (\text{Random error}). \]

This is the formula fitted in ANOVA II (Table 2), with scv-residuals in Fig.3. (The estimated genotype means and 99% confidence intervals are 48.9(46,52) for dHZ, 38.5(36,41) for dWT, and 35.5(33,38) for dKO.)

When we focus the analysis on the shortest-lived cell-types, platelets & granulocytes, the results are less noisy. The ANOVA now gives a smaller residual RMS of 2.6%. The lower noise level reveals the highly significant interaction G×D (P=0.001), which expresses the inequality of the day-130 to day-186 changes in Table 3.

**Table 3.** Mean %b-values that determine the G×D interaction for platelets+granulocytes in experiment II.

<table>
<thead>
<tr>
<th>Day</th>
<th>dWT</th>
<th>dHZ</th>
<th>dKO</th>
</tr>
</thead>
<tbody>
<tr>
<td>130</td>
<td>39.9</td>
<td>49.4</td>
<td>33.5</td>
</tr>
<tr>
<td>186</td>
<td>36.4</td>
<td>47.3</td>
<td>35.8</td>
</tr>
<tr>
<td>Change:</td>
<td>−3.5</td>
<td>−2.1</td>
<td>2.3</td>
</tr>
</tbody>
</table>

The genotype means of %b for the HPP-CFCs at day-255 have the same pattern as at days 130 & 186 but at a lower level. With 95% confidence limits, they are 36.4(29,44) for dHZ, 25.2(18,32) for dWT, 19.3(10,28) for dKO, with dHZ still significantly in the lead (P=0.02).

**Experiment III (22, 57, 93 days)**

We analyse separately the III\(_3\) mice bled on days 22, 57, 93 and the fourteen III\(_2\) mice bled on days 22, 57 that did not survive to day-93. The profusion of significant P-values in ANOVAs III\(_3\) and II\(_2\) indicates that the mean %b values have a complex dependence on genotype, cell-type, and day (which we will not attempt to explore). However, the highly significant M×D interaction in ANOVA III\(_2\) is more than noteworthy. It means that, once we have adjusted for the dependence on G, C, and D, there are significant differences between mice in the jump (up or down) between their %b-value for days 22 and 57. Things are simpler and clearer (as in experiment II) if we focus on the average of the two %b-values for platelets and granulocytes. For this datum, the fitting formula [2] simplifies to

\[ \%b = (\text{Genotype mean}) + (\text{Variation dependent on genotype and day}) \]
\[ + (\text{Random variation for the mouse dependent on day}) \]
\[ + (\text{Random error}). \]

For each mouse and day, we can estimate the term on line [3] of this “model”—call it RVM. Mice jump from being
either above or below the mean for the genotype on day-22 to being either above or below on day-57. With a standard deviation (sd) of 5.3%, these jumps (RVM at day-57 minus RVM at day-22) have a variability that is significantly bigger than what even the residual RMS of 3.2% in ANOVA III₂ (for all cell-types) would countenance. However, this extra variability comes only with the post-FU day-57 values: the sds of RVM are 1.3% at day-22 and 5.2% at day-57, a difference that does not significantly depend on genotype. We suggest that this feature of our data may reflect a post-FU reduction in the numbers of HSCs when they are distinguished by their type Gpi-1 marker—a reduction correlated with the fact that these mice did not survive to day-93. See end of Discussion.

Experiment IV (57 days)

The genotype means in Fig.2(IV) present a complex picture, very different from the day-57 results in experiment III post-FU. The ranking of genotypes now depends on cell-type, with highly variable arithmetic differences whose statistical significance is documented in the ANOVA IV of Table 2.

Experiment V (36 days)

In comparison with experiment IV, the means have a more lawful character. Despite the statistically significant G×C interaction, the ranking of genotypes is the same for all cell-types, with dWT well in the lead.

Overall assessment

We have no explanation other than chance variation for the statistically significant reversal of the ranking of dKO and dWT between days 130 & 131 (with no allowance for selection of this feature, P< 0.0005). To have incorporated a random subexperimental variation in our analyses of the genotype differences would have complicated the presentation of the results—and inhibited the explanatory modelling that it has provoked.

There is a reassuring consistency of the residual RMS-values in the six ANOVAS of Table 2, and an acceptable distribution of scv-residuals in all the ANOVAS, four of which are shown in Fig.3. The range of findings in the five experiments can be crudely summarised as:

- 1st-order genotype differences whose long-term character revealed in experiments I and II contrasts with the mixed bag of differences in the short-term experiments;
- 2nd-order differences between cell-types in experiments I, II, and V (contrasting with bigger differences in experiments III and IV);
- 3rd-order differences such as the interaction of genotype with day in experiment II (which will be taken as our second explanatory challenge).
Discussion (and explanatory modelling)

The mixed bag of short-term results

The observations of short-term (less than 100 days after grafting) repopulations III, IV and V were paradoxical. This is likely to be due to a peculiarity of the deletion of blood-forming cells by busulphan: it deletes HSCs but spares committed progenitors, which continue to form end cells (14). Thus enquiry while such progenitors are still in the race gives the impression of chaos as the progenitors of first one genotype and then another form more end-cells. In (14), host progenitors were still forming lymphocytes at day-161, and the race was not over before day-202. For the present work we attempted to abbreviate the race by housing animals in open cages to allow them to encounter pathogens but are uncertain how much this did so. There may be a slight residue of blood formation by grafted or host progenitors at day-130. Since FU deletes cells in cycle the residual progenitors will be killed by it. Blood formation thereafter, especially of short-lived platelets and granulocytes, then reflects hemopoiesis by grafted HSCs.

Two features of the short-term results in Fig.2 merit speculation:
(a) The discrepancy between the results at day-57 in experiments III and IV (see end of Appendix).
(b) The differences between genotypes in the changes between day-22 and day-36.

For (b), our tentative conjectures are:
(i) For all three genotypes, the %b-values at day-22 are depressed by the then-still-high productivity of host progenitors compared with the moderate involvement of grafted progenitors and HSCs.
(ii) By day-36, host progenitor activity is reduced so that the mean of 52% for WT represents the matching performances of grafted (WT) “host” HSCs and WT donor HSCs (that have not yet been influenced by the supposed progressive deterioration manifested by day-255).
(iii) The dHZ and dKO genotypes are still relatively depressed at day-36 because the demographic influence B invoked in the bipolar model only gradually manifests itself in the productivity of end-cells (compared with that of the “host” WT HSCs which benefit immediately from the directly effective influence A).

Why does heterozygosity win the race for long-term repopulation?

Especially for platelets & granulocytes, the values of %b for 130, 131, and 186 days after grafting should be almost entirely determined by the daughter clones of grafted HSCs, and not appreciably influenced by progenitors in the host at the time of grafting (as the short-term results can be expected to be). The most remarkable and unexpected feature of the data is the competitive advantage of the heterozygous graft in the the long-term dHZ subexperiments—“unexpected” because for single influence (unipolar) biological processes a heterozygous effect is usually intermediate between those of the wildtype and knockout genotypes. However this ranking of genotypes need not hold for bipolar
or multipolar phenomena in which the allele has two or more pathways of influence on the final measurement. The simple play with numbers in Table 4 makes the point with total clarity.

### Table 4. Hypothetical illustration of bipolar influence.

<table>
<thead>
<tr>
<th>Influence A</th>
<th>Influence B</th>
<th>Influence A + Influence B</th>
</tr>
</thead>
<tbody>
<tr>
<td>dWT</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>dHZ</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>dKO</td>
<td>0</td>
<td>10</td>
</tr>
</tbody>
</table>

In our bipolar model to explain the genotype differences in the end-cells stemming from grafted HSCs, influence A is taken to be a direct one—that, on an individual cell basis, a grafted dHZ HSC makes a stochastically smaller contribution to the end-cell compartment at any particular time than does a grafted dWT HSC, while a grafted dKO HSC makes an even smaller contribution. (A contribution is stochastically smaller if, for any given contribution level, the probability of exceeding that level is smaller.) Influence A expresses the supposition that, for an individual HSC, Cx43 is better than the KO allele at making the connections in the stroma that assist hemopoiesis. For influence B, we then suppose that, because of influence A, a WT mouse does not need to maintain as many HSCs as an HZ mouse, which in turn does not have as many (in its fetal liver) as a KO fetus has. In other words HZ mice must have more HSCs than WT mice—an inequality presumably doubly inherited by the KO fetus and a prediction open to experimental testing. Influence B is then an indirect demographic one—the greater number of HZ HSCs than WT more than makes up for their lower productivity, whereas the greater number of KO than HZ are not enough to win out over HZ.

Another marker might help to test the model

All the raw data for this work are available in (23). None of it is informative about the absolute numbers of HSCs that participate in our experiments—and that play a central role in our demographic model. At best a %b-value tells us about relative numbers, and then only with an assumption that HSC clone size is independent of genotype. To get data about absolute numbers, we would consider making use of a developmentally neutral, random binomial marker of the sort invoked by Stone (22). The Gpi-1 marker (a/b) itself is completely confounded with the Cx genotype, which our results have shown to be influential (not “neutral”) in the development of the corresponding clones. This implies that the a/b marker cannot do the job. To obtain data that might be richly informative about the “number” of HSCs of each competing genotype (strictly speaking, about the reciprocal of a carefully defined probability π), it will be necessary to make use of a separate neutral marker, a/β say. For each subexperiment, there would then be four categories of distinguishably marked cells: aα, aβ, bα, bβ. The complementary information alluded to would be extractable from the variances and covariances of the percentages of aβ end-cells among those of type aα and of
bβ end-cells among those of type b.

Conclusion

Detailed statistical analysis of features of the data in our five experiments has revealed regularities that suggest and support a bipolar model for the direct and demographic influences of the connexin43 gene on the efficiency of HSC transplantation. Whether or not the model can be validated by further studies, the greater efficiency of the heterozygous connexin43 genotype may be of clinical value in the treatment of a range of haemopoietic disorders.

Appendix

Tentative explanation of the G×D interaction in Table 3

Without refinement, the bipolar model does not explain the 3rd-order effects for G×D in Table 3, since it does not deal with any question of systematic temporal change such as an interaction with D. We offer the following tentative refinement. The results for experiment II as a whole, which include the %b-values for HPP-CFCs at day-255, suggest that we may be dealing with a combination of (a) a progressive change in the long-term balance between donor and “host” HSCs (favouring the latter) and (b) a temporary blip associated with FU treatment at day-157(186-29).

The progressive change is a decline in the proliferative ability of just the donor HSCs, the same for all three genotypes, showing up as the overall decrease of 12% between day-186 and day-255. It also shows as the 3.5% decrease in dWT in Table 3 between day-130 and day-186. But a change that affected donor HSCs per se would not explain the G×D interaction in Table 3 which says that, although dWT lost 3.5%, dKO gained 2.3% and dHZ lost an intermediate 2.1%. For this, we invoke a temporary blip that favours dKO over dHZ (and dHZ over dWT) and suggest that it may be a repeat performance of the model’s influence B: the numerous dKO HSCs dominate a temporary response to FU treatment (one that is less dependent on Cx43 connections). For a time, dKO plays the major role in meeting the urgent temporary need for proliferation (with the less numerous dHZ HSCs as runners-up). Perhaps a similar argument involving influence B would explain the discrepancy between the results at day-57 in experiments III (with FU) and IV (without FU).

Basic data

Here are the five measurements of %b for each mouse in the order (platelets, granulocytes, red cells, B-cells, T-cells). Repeat bleeds are within [ ]s in order of day. The values of %b for the HPP-CFCs at day-255 are italicised.

References


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