

Up-regulation of the connexin43⁺ gap junction network in haemopoietic tissue before the growth of stem cells

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SUMMARY

The early developmental stages of haemopoiesis are thought to be regulated by paracrine growth factors and by the haemopoietic environment. Are gap junctions involved here? Gap junctions are structures in cell membranes allowing the direct transfer of ions and small molecules between adjacent cells and are known to be involved in development. We have found that although connexin43 gap junctions are rare ($0.00016 \pm 0.0002/\mu\text{m}^2$ tissue) in normal adult mouse marrow their expression is 80-fold higher ($0.0292 \pm 0.0147/\mu\text{m}^2$) in neonatal marrow. One difference between neonatal and adult haemopoietic tissue is that in the latter more haemopoietic cells are dividing. To test if more gap junctions were due to increased division we altered adult blood-formation by mobilizing or destroying end cells - granulocytes and red cells - or by forcing stem cells to divide by making them regenerate an ablated blood-forming system. Mobilizing

end cells had no effect on the number or distribution of gap junctions in marrow but forced stem cell division caused a 100-fold increase in gap junction expression and did so before any recognizable haemopoietic cells formed. There were greater than normal numbers of gap junctions in radio-protected adult mouse marrow. The cells coupled by gap junctions are TE-7⁺ mesodermally derived fibroblasts, STRO-1⁺ stromal cells, and CD45⁺ and CD34⁺ haemopoietic cells. We propose that there is a latent network of Cx43⁺ gap junctions in normal quiescent marrow. In response to events that call for active division of stem cells this network is amplified and coupled to haemopoietic stem cells, perhaps enabling them to divide.

Key words: gap junction, connexin43, blood-formation, 5-fluorouracil, neonatal haemopoiesis

INTRODUCTION

At least two mechanisms are held to regulate blood-formation, paracrine haemopoietic growth factors (see reviews by Metcalf, 1989; Arai et al., 1990), and the effects of cell adhesion molecules and the extracellular matrix on the relationship between haemopoietic cells and the haemopoietic stroma (reviewed by Long, 1992). Blood-formation withstands perturbations so rapidly and successfully, however, that additional mechanisms are likely to be involved.

This paper is concerned with an investigation into the possible role of gap junctions. They are thought to play a part in the development of many tissues (Green, 1988; Warner, 1988; Loewenstein and Rose, 1992). They form specialized regions of intercellular contact, and consist of aggregates of channels that allow the direct transfer between cells of ions or small molecules up to a molecular mass of 1 kDa (Loewenstein, 1981; Warner, 1988). A channel consists of two connexons, one contributed by each of the adjacent cells. Each connexon is made up of six identical protein subunits, connexins (Cx; Zimmer et al., 1987; Milks et al., 1988). As seen by thin-section and freeze-fracture electron microscopy gap junctions have a characteristic appearance (Severs, 1990),

and they can be detected electrophysiologically or by dye-transfer (reviewed by Warner, 1988) when a dye such as Lucifer Yellow (M_r 457), micro-injected into one cell, spreads to another through intercellular junctions.

The presence of gap junctions has often been reported in vitro in haemopoietic model systems (Levy et al., 1976; Porvaznik and MacVittie, 1979; Kapsenberg and Leene, 1979); Allen, 1981; Neumark and Huynh, 1989; Rosendaal et al., 1991; Dorshkind et al., 1993).

Morphological evidence for the presence of many gap junctions in normal adult marrow however is not compelling. Only Campbell (1980, 1982) found them in any number and it is probable that tannic acid, which he used as a fixative, may have been deposited in cell membranes to create the appearance of gap junctions (see discussions by Campbell, 1982; and Watanabe, 1985). Watanabe (1985), who used either 2% gallic acid instead of tannic acid with glutaraldehyde or conventional aldehyde fixatives, found gap junctions between peri-arterial fibroblasts and on fibroblasts in the haemopoietic tissue between sinusoids. Yamazaki (1988; Yamazaki and Allen, 1991) noted rare gap junctions in Sl/Sl^d and C57Bl6 mouse stromal cells. Weiss (1976, 1981), who examined normal rodent marrow extensively, did not note many gap junctions.

Functional assays such as dye-transfer give a different picture and there is an interesting relationship between the incidence of dye-coupling and the length of time that haemopoietic tissue has been out of the animal. In fresh *ex vivo* clumps of bone marrow we observed that dye-transfer takes place between cells in about one clump in 20, but after 48 hours' culture more than eight clumps in ten are coupled and in long-term bone marrow cultures there is extensive dye-coupling (Rosendaal et al., 1991). It is as though removing marrow from bone removes a restraint on the expression of gap junctions. Confocal microscopy reveals that these dye-coupled haemopoietic cells are coupled to the micro-injected cell through the fine processes of certain stromal cells (Rosendaal et al., 1991). In haemopoietic long-term bone marrow cultures, moreover, these stromal cells are dye-coupled to each other and to groups of smaller, round haemopoietic cells. Freeze-fracture electron microscopy confirms that there are numerous gap junctions between cultured stromal cells (Rosendaal et al., 1991). Are these *in vitro* gap junctions just due to an artefactual up-regulation of gap junction formation, a consequence of tissue culture, or are they the remains of a gap-junctional communicating network that is latent but restrained in normal marrow and, if so, which haemopoietic states will release that restraint so that the expression of gap junctions in marrow is up-regulated in the animal? To try and answer these questions we have developed techniques to preserve marrow microanatomy and stain immuno-histochemically large volumes of it for gap junctions.

Eleven mammalian connexins, named by their molecular mass, have been cloned and sequenced (Beyer et al., 1990; Kumar and Gilula, 1992), so site-specific antibodies to synthetic peptides matching unique regions of each connexin can be raised (Milks et al., 1988; Beyer et al., 1989; Harfst et al., 1990). One such antibody (HJ) raised against a unique sequence of Cx43 (Harfst et al., 1990)-labelled gap junctions in undisturbed mouse and human haemopoietic tissue. We found haemopoietic conditions in which their number and distribution differed from normal.

MATERIALS AND METHODS

Animals

B10C mice were used as prescribed by the Animal (Scientific Procedures) Act, 1986.

Normal adult controls

Male and female mice were 10 weeks old or more. They were killed and their long bones placed immediately into fixative (see below). Experiments were repeated three or more times.

Neonatal

These were collected 9, 16 and 24 days after birth.

Treatments to alter haemopoietic demand, end cells

Escherichia coli lipopolysaccharide (0.55:B5, Difco; 25 mg/mouse) was injected subcutaneously to mobilize granulocytes, phenylhydrazine-HCl was injected for three days (Sigma, cat. no. P 7126; 1.0 mg/mouse, pH 7.4) to destroy red blood cells, or mice were bled (0.4 ml) to remove red cells.

Stem cell division

5-Fluorouracil (Chadwick and Rogers, 1972) was injected intra-

venously (Sigma F 6627, 150 mg/kg body weight). Mice were gamma irradiated (9.35 Gy) or radio-protected by the intraperitoneal administration of cytosine arabinoside (Sigma, C1768, 200 mg/kg body weight) three days before 6.5 Gy gamma irradiation.

Perturbing the expression of gap junctions

Susphrine, long-acting adrenaline (Forrest Pharmaceuticals Inc. Missouri), was injected subcutaneously in a dose that did not stress mice (16.7 µg adrenaline). 6-Hydroxydopamine (6-OHDA, Sigma, H 6507) was administered intraperitoneally (100 mg/kg body weight in PBS, twice with an 8-hour interval). Mice were X-irradiated (Marconi, 125 kV, 20 mA, HVL 4mm Al) to compare shielded (5 mm lead) with exposed limbs.

Human marrow

Segments of human trephines obtained for diagnostic purposes were fixed as below.

Immunohistochemistry and gap junction quantification

Bones were segmented and fixed in Zamboni's fixative (2% paraformaldehyde, 0.2% picric acid in 0.1 M phosphate buffered saline, (PBS) pH 7.4) for 1-2 hours, decalcified over seven days in 0.3 M EDTA containing sodium azide (0.02%), pH 7.4, dehydrated through an ethanol and chloroform series, and embedded in wax according to standard procedures. Sections (10 µm) were dewaxed in xylene, rehydrated through an ethanol series and placed into PBS. They were then treated for 10 minutes at room temperature with 0.1% trypsin (Sigma T 8128) in 20 mM Tris-buffer (pH 7.4) containing 0.1% CaCl₂ to re-expose antigenic sites. Sections were blocked with 0.1 M L-lysine in PBS containing 0.1% Triton X-100 for 30 minutes and incubated overnight at 4°C with an anti-peptide antibody (HJ, 1:500 dilution) raised against a synthetic peptide constructed to match a portion (amino acids 131-142) of the Cx43 gap junction protein described by Beyer et al. (1987). Cx43 mRNA has previously been reported in mouse macrophages (Beyer et al., 1991). The secondary antibody was biotinylated donkey anti-rabbit (Amersham RPN 1:250) and the third layer was streptavidin-fluorescein (Amersham RPN 1232 1:250). For full details of the primary antibody see Harfst et al. (1990) and Gourdie et al. (1991). Control labelling experiments were carried out in which immune serum was omitted during the labelling run.

Cx43⁺ gap junctions in rat heart have now been immunolabelled with the primary Cx43 antibody, gold-labelled and localized in transmission electron micrographs (Severs et al., 1993; Green and Severs, 1993).

Appropriate specimens were double-labelled with CD34 (Serotec QBEND/10 1:100), with CD45 (2D1 supernatant 1:1, generous gift of Professor Peter Beverley, London), with TE-7 (Haynes et al., 1984; 1:20, generous gift from Dr Rafaella Schiro, Milan), with STRO-1 (Simmons and Torok-Storb, 1991; supernatant, 1:1, Developmental Studies Hybridoma Bank at the Department of Biology, University of Iowa, Iowa, Contract no. NO1-HD-2-3144 NICHD), with F4/80 (Austyn and Gordon, 1981; supernatant, 1:1, a generous gift from Professor Siamon Gordon, Oxford), with MTS-7 (Godfrey et al., 1990; a generous gift from Dr R. L. Boyd), with E13 161 (Spangrude et al., 1988; Sca⁺, ATCC) and with polyclonal rabbit anti-mouse type IV collagen (Biogenesis, 2150 2288, 1:10,000). The second layer antibodies for these were obtained from Vector and were conjugated with Texas Red, which is optimal for viewing double-labelled images on our confocal microscope.

Image acquisition and analysis

Single images were collected on a Bio-Rad MRC-500 laser scanning confocal microscope (CLSM) using a ×60 objective (Nikon PlanApo, 1.4NA), at zoom ×1.5 and at 256 levels of brightness. One confocal aperture was used throughout.

The number of gap junctions in marrow was assessed by quantifying gap junctions and tissue area. The number of gap junctions was

counted with an algorithm that exploited their compactness and circularity, and their being brighter than surrounding tissue. The technique is based on the computation performed by infant vision (Marr, 1982). The confocal image $I(x,y)$ was convolved with the difference-of-Gaussian function $G(x,y)$:

$$G(x,y) = \frac{s_1}{\sigma_1\sqrt{2\pi}} e^{-\frac{(x^2+y^2)}{2\sigma_1^2}} - \frac{s_2}{\sigma_2\sqrt{2\pi}} e^{-\frac{(x^2+y^2)}{2\sigma_2^2}},$$

which we approximate with a 11×11 local neighbourhood mask. By controlling the scaling factors s_1 , s_2 and the standard deviations σ_1 , σ_2 it is possible to detect gap junctions of a given size. Large objects such as autofluorescing bone give a poor response to the DOG operator.

The convolved image has a characteristic intensity distribution that is easy to threshold. The threshold value t_g is obtained as follows: a least squares regression line is fitted to the early part of the intensity distribution, finding the x -axis intercept t_x of this line and then adding a small constant offset t_c :

$$t_g = t_x + t_c.$$

For each object r , in the binary image, we determine the area a_r , the mean brightness b_r , and circularity c_r (defined as $perimeter^2/area$), and classify the object as a gap junction if:

$$a_{min} < a_r < a_{max}$$

and

$$b_{min} < b_r < b_{max}$$

and

$$c_{min} < c_r < c_{max}$$

where a_{max} , a_{min} , b_{max} , b_{min} , c_{max} and c_{min} are the empirically derived maximum and minimum values for area, brightness and circularity, respectively.

Since tissue without junctions stains weakly it was transformed with greyscale morphological closing (Gonzalez and Woods, 1992), which provided a good threshold between tissue and background so that tissue area could be counted.

Gap junctions and tissue were counted for 10 images that tessellated the most heavily gap-junction-populated region of the specimen. Gap junction density was defined as:

$$\text{gap junction density} = \frac{\text{total number of gap junctions}}{\text{total tissue area}}.$$

Electron microscopy

Thin-section electron microscopy was carried out on control and fluorouracil-treated bone marrow five days after administering the drug. Bone segments were immersion-fixed in 3% glutaraldehyde in 0.13 M phosphate buffer for 3 hours and decalcified over 10 days in 0.1 M EDTA, pH 7.4. The tissue was then post-fixed in 1% OsO₄, en bloc stained with uranyl acetate, dehydrated in a series of ethanols and propylene oxide, and embedded in Araldite. Sections were post-stained in lead citrate and uranyl acetate prior to viewing.

RESULTS

Gap junctions on the processes of cultured stromal cells

Long-term bone marrow cultures were immuno-stained with HJ and scanned. Numerous bright fluorescent dots were found on the processes of stromal cells crossing others (Fig. 1). Since these lay where plaques of gap junctions had been detected by

freeze-fracture and electron microscopy (Rosendaal et al., 1991) they were probably Cx43⁺ plaques. There were also bright dots between stromal and haemopoietic cells in cultures to which fresh marrow had been added a week previously (Fig. 2).

Gap junctions in normal adult mouse marrow

When control adult marrow was stained with HJ there were few bright dots, some were along the junction between bone and haemopoietic tissue, the endosteal-haemopoietic margin, beside the first layer of cells (osteoblasts) between bone and marrow. In normal marrow gap junctions are extremely rare, 0.00016±0.0002 gap junctions per μm² of tissue and osteoblasts are slightly autofluorescent (Fig. 3).

Gap junctions in neonatal mouse haemopoietic tissues

In neonatal marrow there were up to 80-fold more junctions than in controls (Fig. 4); they still line the endosteal-haemopoietic margin in association with osteoblasts but also occupy a ten-cell-deep band extending into the marrow. These are called 'organized, pericellular junctional plaques' here. There are none about the venous sinuses. Most are seen at 16 days (0.023±0.006 per μm², Fig. 5) but the variability in their distribution means that these numbers should be interpreted qualitatively.

Gap junction expression following haemopoietic stresses

End cell treatments

There are no detectable alterations compared with adult controls after these procedures (data not shown).

Effects of forced stem cell division

For 7 days after fluorouracil treatment the amount of haemopoietic tissue in marrow diminishes. After nine days marrow regrows and the bone is filled by 11 days. There is nearly a 100-fold increase in the number of gap junctions in post-fluorouracil marrow, which peaks between days 4 and 6 (Fig. 6). It precedes recognizable haemopoietic cell formation; when that occurs the number of junctions falls rapidly. Many gap junctions lie within five and ten cell diameters of the endosteal-haemopoietic

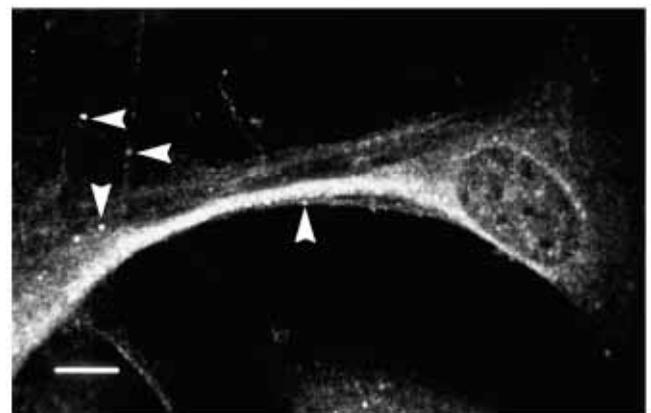


Fig. 1. Immunohistochemical labelling with HJ of Cx43⁺ gap junctions (arrowheads) in cultured mouse haemopoietic stromal cells. Gap junctions are labelled with arrowheads. There is a high background level of auto-fluorescence on these stromal cells. Bar, 10 μm.

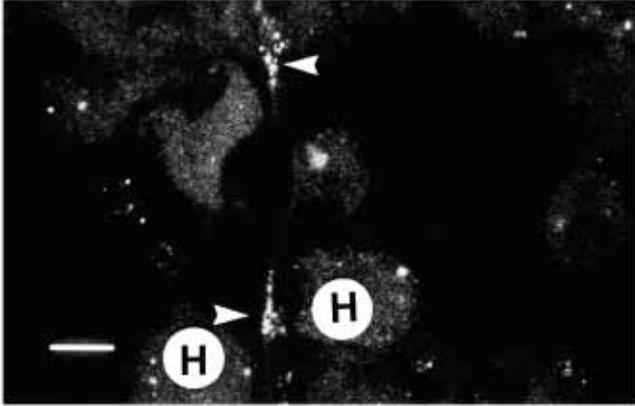


Fig. 2. Immunohistochemical labelling (HJ) of plaques of gap junctions (arrowheads) in long-term bone mouse marrow cultures. Note the many junctions (labelled with arrowheads) close to the haemopoietic cell (H). These plaques may be larger than those in Fig. 1 for functional reasons. Bar, 10 μ m.

margin, but in post-fluorouracil marrow there are also gap junctions deeper in the marrow space, which are arranged in chains along the processes of stromal cells and there are haemopoietic pockets within bone with many junctions in them.

After fluorouracil but not in neonatal marrow there is immuno-stained material within cells. For comparison these are called 'up-regulated, recently formed, intracellular structures' (Fig. 7). The appearance of gap junctions after 9.35 Gy of irradiation does not differ from that after fluorouracil.

Peptide competition

To test that all immuno-stained material in post-fluorouracil marrow represented Cx43 sequences this marrow was immuno-stained with HJ antibody to which excess peptide (0.015 μ g peptide/100 μ l serum) was added prior to incubation. This is the middle of a range (0.0015 to 0.15 μ g) shown to be effective in eliminating specific labelling in rat heart that expresses large amounts of Cx43 (Gourdie et al., 1992). No labelling was observed in post-fluorouracil marrow when the antibody had been exposed to excess peptide, consistent with HJ antibody staining intracellular gap junction sequences.

Gap junctions in human marrow after combined chemotherapy

To test if Cx43⁺ gap junctions also formed in human marrow, human trephine samples were immuno-stained. These were collected for diagnostic or therapeutic purposes from patients with haemopoietic malignancies regenerating their marrow

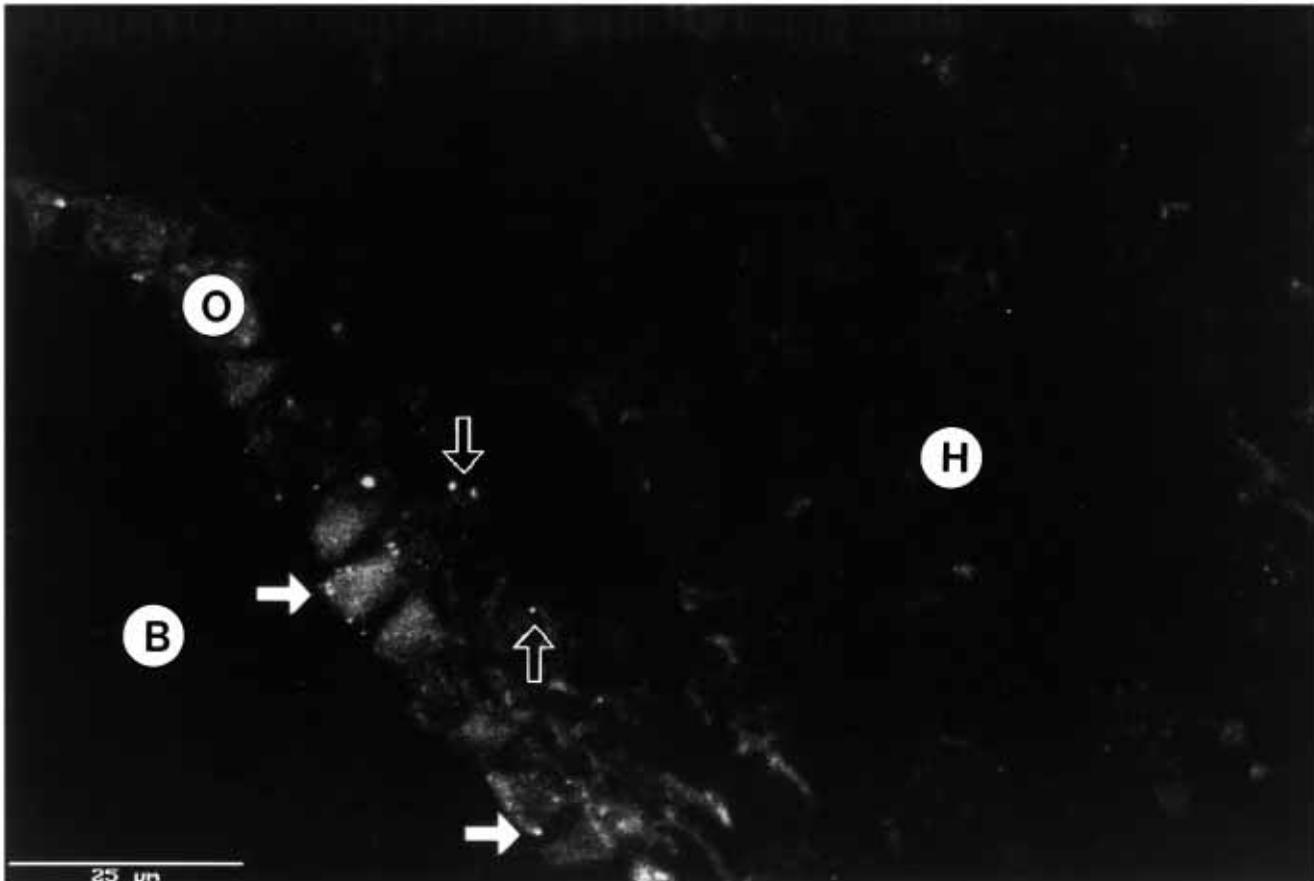


Fig. 3. Immunohistochemical labelling (HJ) of normal adult B10C marrow. Surrounding bone, which is out of focus, is labelled B, the layer of osteoblasts, O, and haemopoietic tissue, H. Two kinds of gap junction are arrowed. Filled arrows point to junctions between osteoblasts, which are lightly autofluorescent, at the endosteal-haemopoietic margin. Open arrows denote junctions between deeper cells that are not autofluorescent and which lie in the haemopoietic part of the marrow. Bar, 25 μ m.

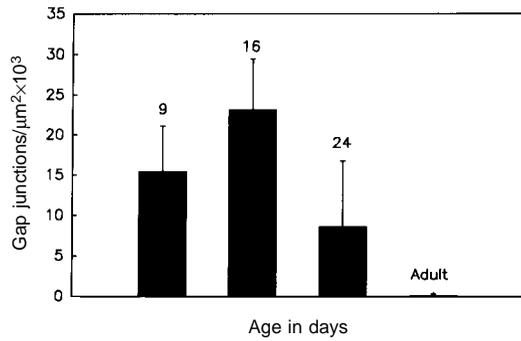


Fig. 4. The number of gap junctions ($\times 1000$) \pm s.d. per μm^2 of neonatal B10C marrow 9, 16 and 24 days after birth. The highest number is more than 80-fold that found in normal marrow. The large s.d. bars reflect the variable distribution of junctions in this tissue. The age in days is indicated over the s.d. bars.

after combined chemotherapy a month previously. There are Cx43⁺ gap junctions in these. They are organized peri-cellular junctions rather than the up-regulated, recently formed, intracellular structures seen in post-fluorouracil marrow.

Gap junctions in radio-protected mouse marrow

Immediately after sublethal irradiation and for the next two days there were more than twice as many gap junctions in radio-protected marrow as in controls. During the next four days radio-protected mice sustained less tissue damage than controls (so that their gap junction count per area of tissue was less than that of controls) and this was accompanied by a more sustained wave of gap junction formation.

Regulation of gap junction formation

Hormonal regulation of haemopoietic gap junctions

The number of gap junctions increases within 24 hours of the administration of long-acting adrenaline and remains higher than controls for 6 days (data not shown). These are up-regulated, recently formed, intracellular structures. Inconstantly the number of gap junctions increases 24 hours after 6-OHDA, consistent with the sudden release of stored catecholamines.

Local regulation of gap junction expression

Mice were exposed to 6.5 Gy of X-irradiation and the number of gap junctions in whole body irradiated femora was compared with that of mice whose left femora were shielded and whose right was exposed. Gap junctions in shielded

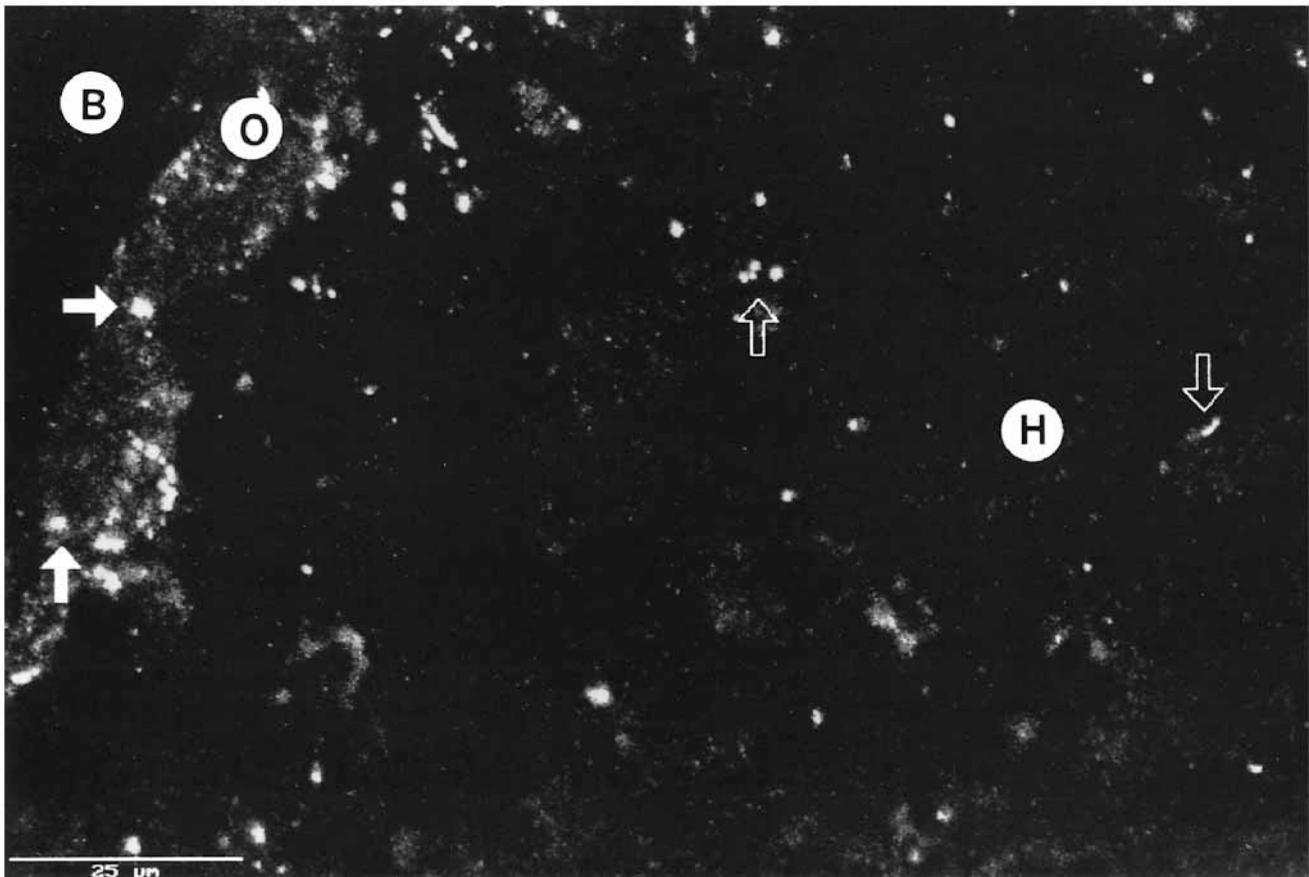


Fig. 5. Immunohistochemical labelling (HJ) of neonatal (9 days old) ex vivo B10C marrow. As in Fig.3, bone is labelled, B, osteoblasts, O, and haemopoietic tissue, H. Two kinds of gap junctions are arrowed. Junctions between osteoblasts at the endosteal-haemopoietic margin are marked with filled arrows. Organized, pericellular junctions deeper within haemopoietic marrow are marked by open arrows. Bar, 25 μm .

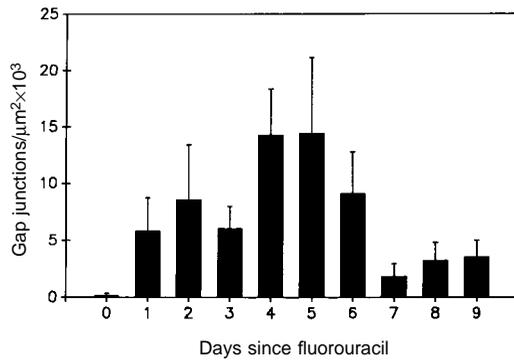


Fig. 6. The number of gap junctions ($\times 10^3$) \pm s.d. on a μm^2 of B10C marrow at intervals after treating mice with 5-fluorouracil.

femora were normal, and there were more gap junctions in whole body irradiated and exposed femora.

Electron microscopic confirmation of haemopoietic gap junctions

HJ labelling of gap junctions was confirmed by electron microscopy. In marrow of controls no gap junctions were found. Five days after fluorouracil, there were several gap

junctions within a single grid square (200 mesh grid). They vary up to $0.7 \mu\text{m}$ in length and most lie between osteoblasts along the endosteal-haemopoietic margin, as shown in Fig. 7. Many are also found between stromal processes contacting osteoblasts, between stromal processes, between stromal processes and early haemopoietic cells further into marrow (recognised by their rounded shape and size) and between haemopoietic cells alone (Fig. 8). The junctions have a typical pentalaminar structure (detail) although the bilayer structure of the cell membrane is difficult to distinguish in this tissue.

Cells coupled by Cx43⁺ junctions in human marrow include TE-7⁺ mesodermally derived fibroblasts, STRO-1⁺ stromal, and CD45⁺ and CD34⁺ cells. In post-fluorouracil and neonatal marrow Cx43⁺ junctions are found on cells beside Sca⁺ cells and rarely on MTS7⁺ cells (thymic cortical epithelial marker). F4/80⁺ cells and mouse collagen-IV⁺ stromal processes are not Cx43⁺.

DISCUSSION

Our major finding, that the number of gap junctions is 80- to 100-fold higher in neonatal marrow and adult marrow during forced stem cell division, is based on our immunological detection of Cx43⁺ gap junctions. The evidence that HJ detects

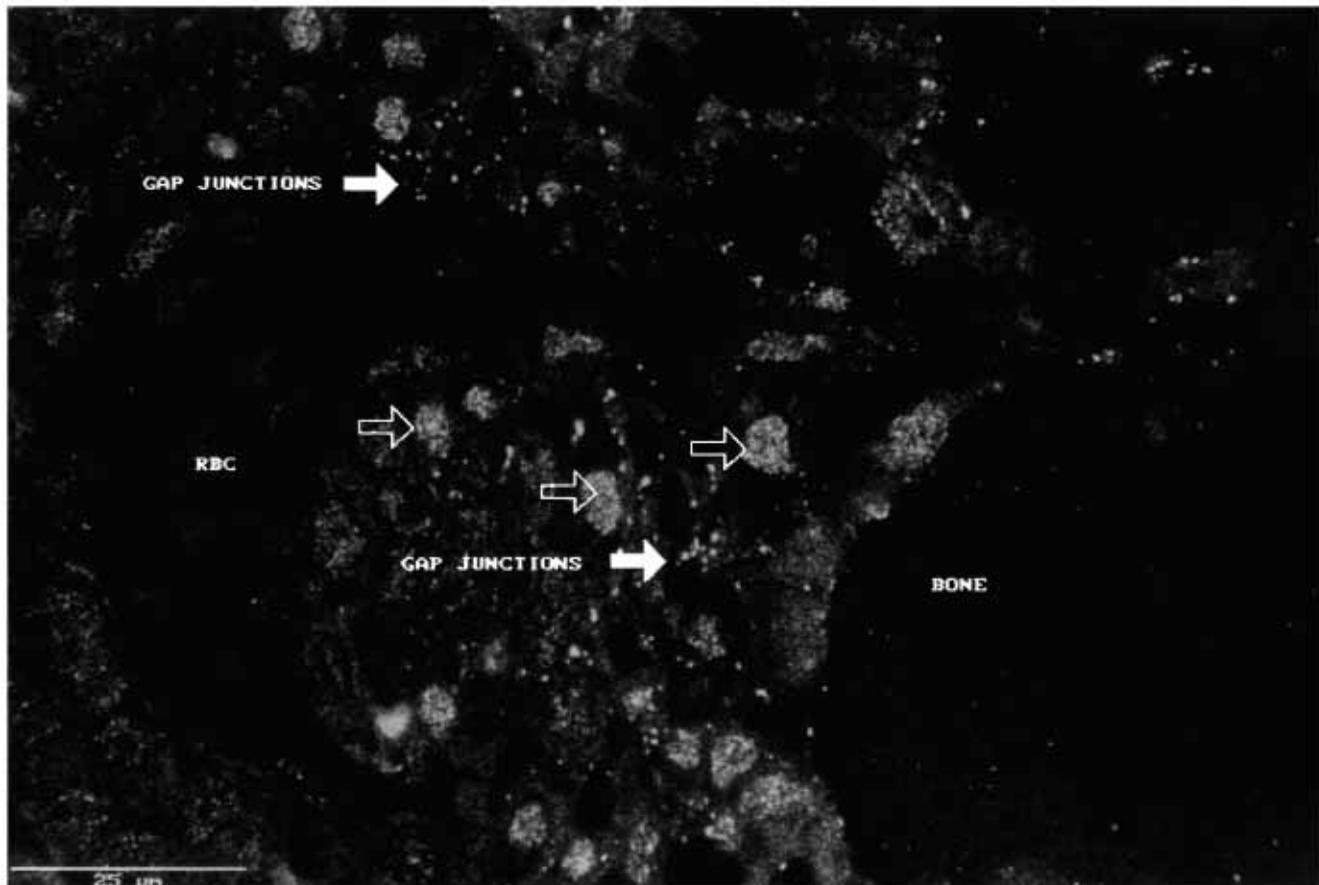


Fig. 7. Immunohistochemical labelling (HJ) of ex vivo B10C marrow collected 5 days after treating the animal with fluorouracil. Typical up-regulated, recently formed gap junctions are marked with filled arrows. Intracellular immuno-stained material is marked with open arrows. Bone and red cells in a venous sinus are also indicated. Bar, $25 \mu\text{m}$.

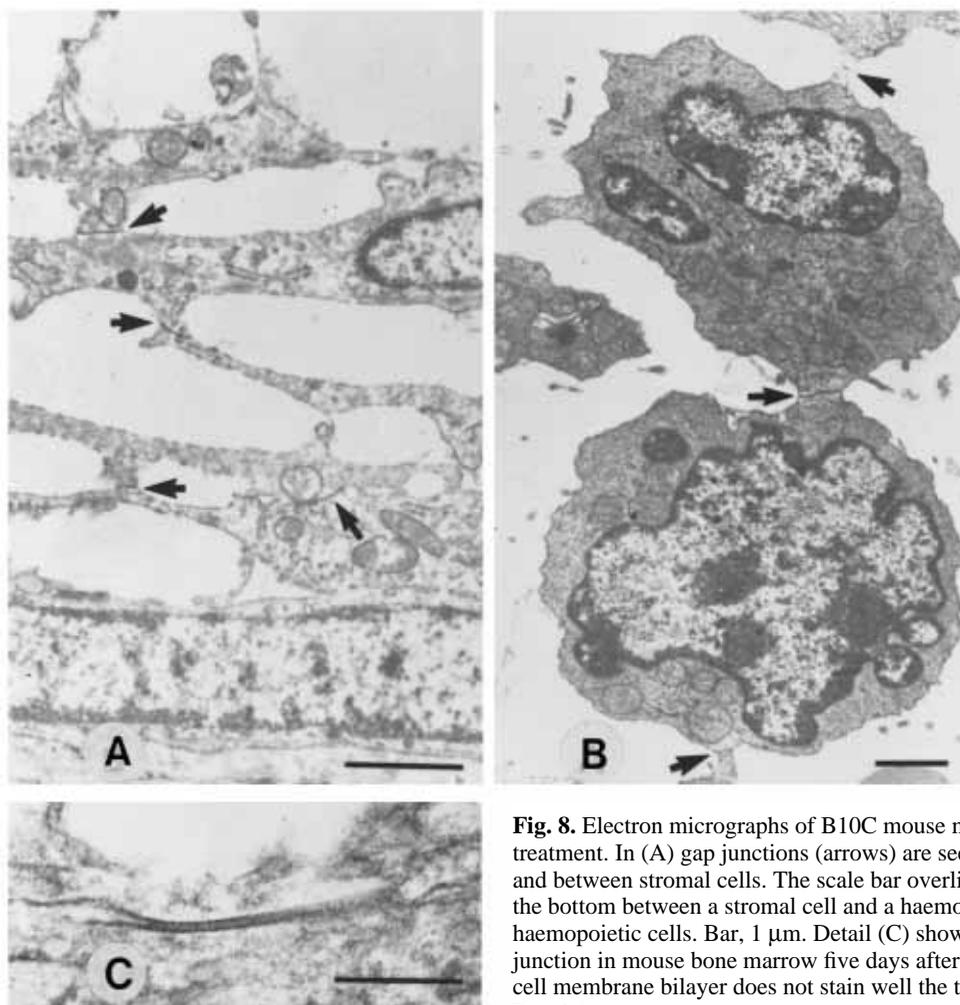


Fig. 8. Electron micrographs of B10C mouse marrow five days after fluorouracil treatment. In (A) gap junctions (arrows) are seen between an osteoblast and a stromal cell and between stromal cells. The scale bar overlies bone. In (B) a gap junction is arrowed at the bottom between a stromal cell and a haemopoietic cell and, in the middle, between haemopoietic cells. Bar, 1 μm . Detail (C) shows a higher magnification view of a gap junction in mouse bone marrow five days after fluorouracil treatment. Even though the cell membrane bilayer does not stain well the typical gap junction structure is evident. Bar, 0.2 μm .

Cx43⁺ gap junctions between cells is 5-fold. Firstly, HJ identifies a sequence that is unique for Cx43 (Beyer et al., 1990; Harfst et al., 1990). Secondly, in cardiac myocytes, gap junctions immunogold-labelled with HJ colocalize with junctions identified by conventional transmission electron microscopy (Severs et al., 1993; Green and Severs, 1993). Thirdly, peptide competition of HJ in marrow and rat heart (Gourdie et al., 1992) abrogated antibody binding to Cx43 gap junctions. Fourthly, freeze-fracture electron microscopy and HJ detect gap junctions in the same site on the processes of cultured stromal cells; and finally, in post-fluorouracil marrow detection of gap junctions with TEM correlates with detection of gap junction by the antibody and there are numerous gap junctions whereas there are none in normal marrow. However, HJ appears also to detect intracellular Cx43 sequences that are not yet present in the membrane. This makes possible a distinction between functional, organized, pericellular gap junctions and up-regulated, recently formed, intracellular structures. The former are found in neonatal haemopoietic tissue and in human marrow collected a month after combined chemotherapy. The latter are formed after stress, fluorouracil or adrenaline. The fact that organized junctions form in human marrow a month after injury argues that they may form from up-regulated, recently formed material.

Administration of catecholamines or 6-OHDA increased the number of gap junctions. The observation was in agreement with findings with rat C-6 glioma cells (Radu et al., 1982). 6-OHDA has a biphasic action on catecholamine levels. First, it binds to catecholamine storage granules so they release stored catecholamine. Then it blocks catecholamine release until new granules are synthesized (Jonsson, 1980).

Gap junctions allow the direct transfer of regulatory molecules (<1 kDa) between cells and are thought to play an important role during development (see, for example, Warner et al., 1984; Fraser et al., 1987). Several lines of evidence indicate that they also play a role in allowing the transfer of growth-regulating molecules between cells (for review, see Loewenstein and Rose, 1992). In proliferating hepatocytes there are fewer gap junctions than normal (Dermietzel et al., 1987), but the incorporation of connexin genes into transformed cells normalizes their level of intercellular communication and subsequently they grow (Mehta et al., 1991). Both epidermal growth factor and platelet-derived growth factor have been shown to modulate communication between cells (Loewenstein and Rose, 1992), so a role for gap junctions in regulating haemopoiesis would not be unexpected.

This report provides two sorts of information about increased expression of gap junctions: when the junctions form

and where. Most gap junctions are found in neonatal haemopoietic tissues, which are laying down the blood-forming system by the proliferation of stem cells. In neonatal and up-regulated adult marrow most gap junctions lie close to bone, near the endosteal-haemopoietic margin, whence surviving stem cells will regenerate blood-formation after administration of cytotoxic drugs (Shackney et al., 1976; Rosendaal et al., 1979; Hodgson et al., 1982; Rosendaal and Adam, 1987; Bartelmez et al., 1991).

We propose that in normal marrow there is a latent CX43 gap junction communicating network between certain stromal cells. This network is up-regulated before stem cells divide and then embraces haemopoietic as well as stromal cells. Possibly this network is involved in the transfer of information between stromal and stem cells to enable these developmentally early cells to divide. There is no evidence for the network in foetal liver and in this respect hepatic blood-formation differs from the adult. There may be traces of the network in semi-solid cultures of canine macrophages (Porvaznik and MacVittie, 1979) and human T-cells (Neumark and Huynh, 1989), and the network is extensive in long-term bone marrow cultures (Rosendaal et al., 1991).

In studies in progress we are classifying haemopoietic cells coupled by gap junctions and examining the effects in model blood-forming systems of cultures over stromal cells coupled to different extents.

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