Islets of Langerhans: Dye Coupling Among Immunocytochemically Distinct Cell Types

Abstract. *Cells in isolated rat islets of Langerhans were microinjected with Lucifer Yellow CH in a medium containing 16.7 millimolar glucose. Dye was rapidly transferred from the injected cell to neighboring islet cells without specificity with regard to the immunocytochemical identity of either the donor or the recipient cells. The transfer of dye between the islet cells (types A, B, and D) demonstrates homologous and heterologous cell coupling in a system where the normal proportions and relationships of the cell types are maintained.*

Gap junctions are membrane specializations that are thought to provide the structural basis for the transfer of ions (ionic coupling), and low molecular weight molecules (dye and metabolic coupling) from one cell to another in a number of vertebrate and invertebrate systems [for reviews, see (*1, 2*)]. Ultrastructural studies have identified these permeable junctions between insulin-producing B cells, and between adjacent B cells and glucagon-producing A cells in the islets of Langerhans of several species (*3–5*). The presence of these junctions may account for the synchronized electrical activity between B cells of isolated mouse islets (*6*), and for the transfer of glycolytic substrates (*7*), labeled nucleotides (*8–9*), and fluorescent dye molecules (*7, 10*) between cells in monolayer culture derived from adult or fetal rat islets. However, the specific function of these junctions with regard to cellular activity is not known.

There has been no demonstration of the intercellular transfer of small molecules between cells of an intact islet in which the relationships and proportions of each of the different cell types is maintained. In the present study, we demonstrate dye-coupling between cells of the isolated rat islet by microinjecting a fluorescent dye, Lucifer Yellow CH, into donor cells and observing its transfer to neighboring cells.

Details of the islet isolation and microinjection procedure, and techniques for visualizing both the fluorescent dye and immunocytochemical stain in the same tissue section have been described (*11*). Briefly, the islets of Langerhans were isolated from the pancreas of female Holtzman rats (225 to 250 g) according to a modification (*12*) of the collagenase isolation technique of Lacy and Kostianovsky (*13*). Isolated islets were attached to poly-L-lysine coated petri dishes (*14*), and allowed to recover for 45 minutes at 37°C in Krebs-Ringer bicarbonate buffered medium (*p*H 7.4), containing bovine albumin (2 mg/ml) and glucose (2.8 m*M*). After the recovery period, medium was exchanged with fresh medium containing 16.7 m*M* glucose and the islets were incubated for 90 minutes at 37°C. Cells at the islet periphery were microinjected (*15*) at random with Lucifer Yellow CH, a low molecular weight and highly fluorescent probe that will not permeate the nonjunctional membrane of a viable cell (*16*). Shortly after injection, the islets were fixed with 4 percent paraformaldehyde in phosphate buffered saline, embedded in Epon 812, and serially sectioned at a thickness of 1 μm. After removal of the resin (*17*), sequential sections [and control sections (*18*)] were stained to determine the distribution of insulin, glucagon, somatostatin, or pancreatic polypeptide (*19*) by means of the indirect immunofluorescent technique of Coons (*20*), with a rhodamine-conjugated secondary antibody (Cappel).

Thirty peripheral cells of glucose-stimulated islets were injected with Lucifer Yellow CH. On the basis of immunocytochemical staining, 56.7 percent were identified as insulin-producing B cells, 33.3 percent were glucagon-producing A cells, and 10.0 percent were shown to be somatostatin-producing D cells. To date, no pancreatic polypeptide cells have been injected, presumably because of the low density of this cell type in the predominantly dorsally derived islets that we studied (*21*).

As with islet cells in monolayer culture (*7, 9, 10*), we found B cells of the intact islet to be capable of transferring small molecules (in this case, dye molecules) to neighboring B and non-B cells (Fig. 1, I to L; Tables 1 and 2). In addition, we observed the transfer of Lucifer Yellow from injected A and D cells to several of the possible recipient cell types in the peripheral region of the islets (Fig. 1, A

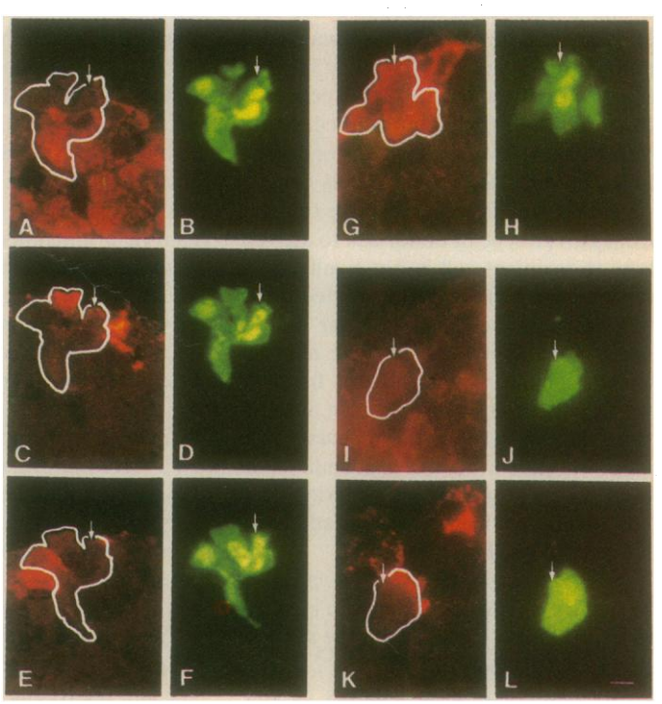

Fig. 1. Lucifer Yellow dye can be transferred within seconds from the microinjected cell (arrows) to neighboring cells in glucose-stimulated, isolated islets. Sections (1 μm) from three islets are shown to demonstrate coupling between peripheral islet cells. Only the immunocytochemically stained sections necessary for the identification of the dye-coupled cells are shown. In the first islet (A to F), a rhodamine-conjugated secondary antibody identifies cells that produce insulin (A), glucagon (C), and somatostatin (E). Corresponding Lucifer Yellow–containing cells may be identified in the same sections (B, D, and F, respectively). (For ease of identification, dye-coupled cells have been outlined in photographs of the immunofluorescent stain.) A comparison of dye-coupled cells with the immunocytochemical stains reveals heterologous cell coupling involving two B cells (bottom two cells in A and B), two A cells including the injected cell (upper center and at arrow in C and D), and one D cell (upper left in E and F). In a section from the second islet (G to H), homologous cell coupling is observed, with dye transferring from an injected A cell (arrow) to four recipient A cells (G, antibody to glucagon; H, dye-containing cells). In the third islet (I to L), an injected B cell (arrow) (I, antibody to insulin; J, dye-containing cells) transfers dye to an A cell (K, antibody to glucagon; L, dye-containing cell). In most instances it was not possible to determine the exact path that the dye took in traveling from one cell to another. However, the microinjected cell was determined by comparing photographs taken immediately after the injection with those taken prior to resin removal. The injected cell almost always contains the greatest amount of dye, and thus appears more fluorescent than its dye-containing neighbors. (×930. The bar in L represents 5.5 μm.)

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Table 1. Percentage of cell types in dye-coupled pairs. The dye-coupled cells were classified according to donor and recipient cell types. The percentage of each particular donor-recipient combination was calculated from the number of times that the combination was observed and the total number of coupled pairs consisting of the same donor cell type. A given donor was sometimes involved in more than one dye-coupled pair (see Table 2). Thus, 30 donors resulted in the 37 dye-coupled pairs indicated in the table.

Recipient cell type	Donor cell					
	A		B		D	
	Percentage	Number of times observed	Percentage	Number of times observed	Percentage	Number of times observed
A	42.9	6	20.0	4		
B	50.0	7	70.0	14	66.7	2
D	7.1	1	10.0	2	33.3	1

Table 2. The number of recipients per donor. For each donor-recipient combination, the mean number of recipients per donor (± 95 percent confidence interval where appropriate) was calculated by dividing the total number of recipients by the number of donors in the particular category. The number of times that a donor-recipient combination was observed is included as in Table 1.

Recipient cell type	Donor cell					
	A		B		D	
	Recipients per donor	Number of times observed	Recipients per donor	Number of times observed	Recipients per donor	Number of times observed
A	1.8 ± 1.1	6	1.7 ± 1.1	4		
B	2.1 ± 0.6	7	1.8 ± 0.3	14	1.5 ± 3.1	2
D	1	1	1	2	1	1

to L, and Tables 1 and 2). Out of 30 injections, the most frequently observed groups of dye-coupled cells included either B cells (36.8 percent) or A and B cells (33.3 percent), the two cell types which together comprise about 95 percent of the rat islet (*21*). Transfers confined to only A cells (10.0 percent) or to D and B cells (13.3 percent) were less frequent, and in only one case was there transfer involving only D cells (3.3 percent) or A, B, and D cells (3.3 percent). If we assume that the injected cells represent the proportion of the donor cell types in the islet periphery, it can be shown that the frequency of dye transfer from a given type of donor cell to a given type of recipient cell is not significantly different from the results expected if the transfer between any particular donor-recipient cell combination were nonspecific (calculations not shown). In other words, the transfer of dye from a donor to a particular cell type appears to be more dependent on the probability that the two cells are in contact with each other than on which particular cell combination is involved.

Around the periphery of isolated islets, dye-coupled cells appear to be organized into small groups, with the average number of recipient cells being 2.5. It is unclear whether these clusters of coupled cells are indicative of the way that islet cells interact in vivo, or whether they represent only a fraction of the coupled cells that normally exist, but which are not observed because of technical limitations (*12, 22*).

Data from seven injections into the central core of whole islets indicate that there is a higher incidence of recipient B cells per donor in this region (5.4 ± 1.9, $P < .05$, $N = 7$, where N = the number of donor B cells) than at the periphery (1.8 ± 0.3, $P < .05$, $N = 14$). These observations are somewhat surprising in view of the finding of Meda *et al.* (*23*) that peripheral B cells have twice as many gap junctions per unit membrane as those located in the islet core. Although we have no explanation for the differences in the two regions, our data suggest that an increase in the number of junctions in going from the center to the periphery of the islet does not result in an increase in the number of dye-coupled cells. More information on the speed of dye transfer and the size of the recipient cells in the two regions will be needed before it will be possible to understand the significance of variations in the number of gap junctions.

There is evidence that as B cells are stimulated to secrete insulin, both the size and the frequency of their gap junctions increase (*24*) as the insulin content of the cells is depleted (*25*). Our studies of the effects of incubation conditions on the coupling capabilities of each of the cell types suggest that islet cells are less frequently dye-coupled in the presence of low glucose (2.8 m*M*) than at high glucose levels (16.7 m*M*), though we have observed some coupling between A, B, and D cells in extremely low glucose concentrations (1.4 m*M*). The increased dye-coupling at high concentrations of glucose is observed for A and D cells as well as for the B cells, although the increased number of recipient A cells may be at least partially attributable to the coupling of this cell type with peripherally located, stimulated B cells. It is interesting that the A cells appeared to increase their communicating abilities in these studies despite the inhibitory effect of high glucose on glucagon secretion (*26*). These observations are consistent with the idea that glucose stimulates changes uniformly throughout the islet, with the entire islet or cell groups within the islet responding as a functioning coordinated unit.

Our results show that Lucifer Yellow CH can diffuse from one cell to another in the intact islet, presumably through gap junctions. Islet cells are responsible for the production and release of hormones essential for the regulation and control of blood glucose concentrations (*27*). Though we can only speculate on the role of these junctions in this system, our results further support the suggestion (*5*) that they may provide a pathway for the transfer of a signal molecule to which islet cells could respond with synchronized hormonal output. Thus, the secretory activity of the cells could be rapidly and efficiently controlled in response to changes in the circulating levels of nutrients and hormones.

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12. The proteolytic digestion of pancreatic tissue during islet isolation may alter the islet cell gap junctions [M. Shimo and F. Clementi, *J. Ultrastruct. Res.* **59**, 101 (1977)]. To avoid perturbing the cells, especially those located at the periphery, we used minced pancreas digested with only 1 mg of Sigma type V collagenase per milliliter of pancreas. Islets were then gently hand-picked from the surrounding acinar tissue. This isolation procedure does not appear to alter the peripheral cell coupling because the injected cells that were exposed on the islet surface and those protected by several layers of cells exhibited the same patterns of coupling.
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15. Glass micropipettes were filled with a 4 percent aqueous solution of Lucifer Yellow CH (W. Stewart, National Institute of Arthritis, Metabolism, and Digestive Diseases, Bethesda, Md.). The dye was passed from the electrode with negative current pulses (7 to 9 nA). When it was questionable whether the electrode had penetrated only one cell during the injection (as determined by problems during the injection or from photographs taken prior to resin removal indicating more than one highly fluorescent cell), the islet was discarded.
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18. Two types of controls were carried out. In the first, normal serum was substituted for the primary antibody. In the second, antibodies were preabsorbed with their corresponding antigen (100 μg/ml) prior to application to sections. In both cases, controls were negative with no specific binding of the secondary antibody to the sections.
19. Primary antibodies were kindly donated by G. Bauer (guinea pig antibody to insulin), S. Erlandsen (guinea pig antibody to glucagon), R. Elde (rabbit antibody to somatostatin), and R. Chance (rabbit antibody to pancreatic polypeptide).
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22. There are limitations in attempting to quantitate the number of dye-containing cells in fixed and embedded tissue. Background fluorescence from the embedding resin (*16*), fading of the dye, or low levels of dye molecules in some of the cells may prevent detection of all of the coupled cells. Therefore, negative results (that is, the lack of visible dye transfer) from either a group of cells or from an individual injected cell need to be interpreted carefully. We report here only positive dye-coupling results, although it should be noted that we were unable to detect any dye transfer from four additional injected cells during the 5-minute injection periods. We believe it is possible to make fairly accurate comparisons of the effects of incubating conditions on the dye-coupling capabilities of the cells in this system if the preservation of the tissue is the same in all cases. To avoid confusion, photographs were taken immediately after islet injection, prior to resin removal, and after resin removal and immunocytochemical staining for comparison. Throughout the procedure, Lucifer Yellow remained in the tissue. In all instances the number of cells that appeared to contain dye prior to resin removal was the same as that after resin removal, and these values corresponded to the number of cells that appeared to be involved in the transfer during the dye injection.
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Further Heterogeneity of Human α Interferon mRNA Species

Abstract. *Translationally active (in* Xenopus *oocytes) human α interferon (IFN) messenger RNA's (mRNA's) derived from Sendai virus–induced leukocyte cultures display a bimodal distribution of RNA lengths on electrophoresis through agarose-CH_3HgOH gels. The major population (α_S) consists of mRNA of length 0.7 to 1.4 kilobases, while the minor population (α_L) consists of RNA of length 1.6 to 3.5 kilobases. Induction of human leukocytes in the presence of 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB; 100 micromolar) appears to inhibit the accumulation of IFN-α_S and to enhance that of IFN-α_L mRNA's (average length about 1.8 kilobases in preparations from DRB-treated cells). Interferons derived from the α_S mRNA's represent the group of previously recognized α interferons while the α_L interferons are distinguishable from this group by their lower heterospecific activity on bovine cells compared to human cells, their apparent slower mobility in sodium dodecyl sulfate–polyacrylamide gels, and their apparent heteroclitic response toward an antiserum to IFN-α.*

Recent advances in the molecular cloning of human interferons (IFN's) and their complementary DNA (cDNA) and chromosomal DNA sequences have provided detailed information about the structure of some human interferon α and β genes (*1–3*). Several investigators (*2*) have cloned and characterized at least eight distinct cross-hybridizing species of cDNA derived from sucrose-gradient enriched 12*S* interferon messenger RNA (mRNA) obtained from either virus-induced human leukocyte or virus-induced human lymphoblastoid cell cultures and have also characterized the structure of interferon genes which correspond to these species. Briefly, these genes do not have introns and appear to correspond to mature polyadenylated mRNA's approximately 0.8 to 1.4 kb in length (collectively referred to by us as IFN-α_S mRNA's). In our present study, we used RNA electrophoresis under stringent denaturing conditions through

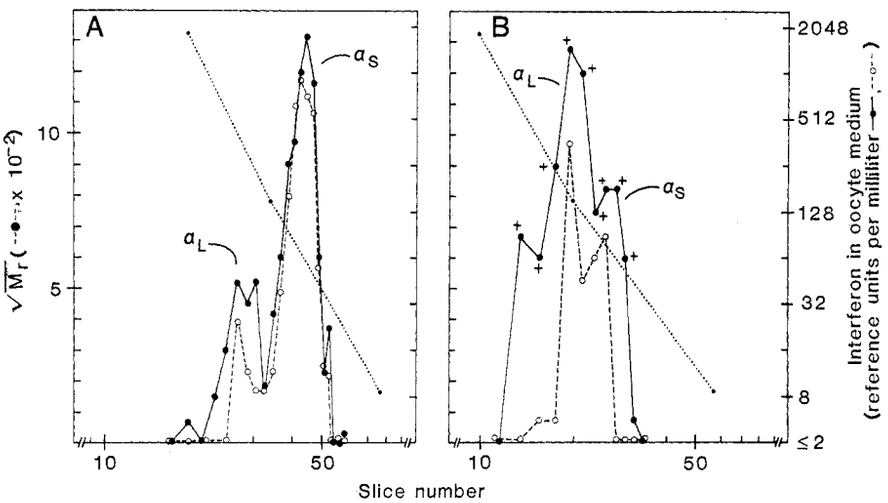

Fig. 1. Agarose-CH_3HgOH gel electrophoresis of IFN-α mRNA's induced by Sendai virus in human leukocyte (peripheral blood buffy coat) cultures in the absence (A) or presence (B) of DRB. Polyadenylated RNA (*11*) was extracted from interferon-primed (100 reference units per milliliter; 2 hours at 37°C) Sendai virus–induced (150 hemagglutination units per milliliter) human leukocytes (~ 5×10^6 cells per milliliter) (*5*) at various times up to 10 hours after the beginning of induction and was pooled. In (B), DRB (Calbiochem; 100 μ*M*) was present from 1 to 2 minutes prior to the addition of Sendai virus until the time of cell harvest. The RNA [23 μg derived from 2.5×10^9 cells in panel (A) and 15 μg derived from 1.6×10^9 cells in (B)] was mixed with ^{32}P-labeled cytoplasmic RNA from HeLa cells; it was denatured in 10 m*M* CH_3HgOH and was then subjected to electrophoresis through a 1.5 percent (A) or 2 percent (B) agarose–10 m*M* CH_3HgOH tube gel (11 by 0.6 cm) (*11*). Adjacent 1-mm gel slices were pooled in pairs; the RNA was eluted and dissolved in 10 μl of sterile, distilled water; and 1 μl of each sample was given by microinjection to groups of *Xenopus* oocytes (NASCO) incubated in 0.2 ml of Barth's medium (*11*). Approximately 24 hours later the interferon content of the oocyte incubation medium was assayed on human cells (GM 2504 or GM 2767, ●) or bovine cells (EBTr, ○) in a semimicroassay of cytopathic effect inhibition; vesicular stomatitis virus was the challenge virus and the result is expressed in terms of the 69/19 or G-023-901-527 NIH reference standards for human interferon. The RNA length was calibrated with marker 28*S*, 18*S*, and 4*S* RNA (dotted line). The interferon activity on human cells was completely neutralized by antiserum to IFN-α (*12*) but was unaffected by antiserum to IFN-β_1 (*13*) (indicated by plus symbols).

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