Interferon-Mediated Induction of la Antigen Expression on Isolated Murine Whole Islets and Dispersed Islet Cells

J. R. WRIGHT, JR., P. E. LACY, E. R. UNANUE, C. MUSZYNSKI, AND V. HAUPTFELD

SUMMARY

Islets from male B10.BR mice (H-2^k) were isolated by the collagenase technique, handpicked with a Pasteur pipette, and incubated (either intact or after dispersion with Dispase) for 0, 3, 5, 7, 10, or 14 days in tissue culture medium supplemented with either lymphokine supernatants or recombinant murine interferon-γ. Islets and single cells were examined for IA^k molecules by use of indirect immunofluorescence. la-positive islet cells were identified on the surface of islets incubated with 5–10% lymphokine for >4 days or with 10, 100, or 1000 ng/ml interferon for >6 days. Islets incubated in unsupplemented medium were la negative. Incubation with 5% lymphokine induced la expression on 10–40% dispersed islet cells cultured for >9 days. Dual immunofluorescent staining for la and insulin revealed that la-positive cells included both β- and non-β-cells.

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Class II major histocompatibility complex (MHC) molecules (IA antigens) play a major role in the initiation of immune responses (reviewed in ref. 1). Important functions include presentation of soluble antigens to helper T lymphocytes and stimulation of alloreactivity. Class II MHC antigens are expressed on B lymphocytes, activated T lymphocytes, activated macrophages, and the interdigitating or dendritic cells of various organs. In recent years it has become apparent that products of activated T cells [lymphokines (LKs)] can induce class II molecule expression on many other cell types. This effect is mediated by interferon-γ (IFN-γ). The functional significance of class II expression in nonlymphoid cells is not known.

Recently, aberrant la antigen expression has been implicated in the pathogenesis of several autoimmune endocrine disorders. Endocrine parenchymal cells do not usually express class II molecules. However, several groups have reported HLA-DR (class II) molecule expression on follicular cells in Hashimoto’s thyroiditis, Graves’ disease, and other autoimmune thyroid diseases. Pujol-Borrell et al. demonstrated that human thyroid follicular cells can be induced to express HLA-DR antigens by culturing with mitogens, an effect that is presumed to be mediated by IFN-γ.

Although pancreatic islet parenchymal cells do not constitutively express class II MHC molecules, these molecules are present on passenger leukocytes and dendritic cells within islets and are believed to be responsible for rejection of islet allografts in experimental animals.

Because type I insulin-dependent diabetes mellitus (IDDM) has long been temporally associated with vague histories of antecedent viral infections, Bottazzo et al. suggested the following mechanism and sequence of events for IDDM. A viral infection involving β-cells may result in localized IFN production, induction of HLA-DR expression on β-cells, presentation of autoantigens, induction of autoimmune T cells, and activation of effector T and B cells against β-cell autoantigens. In support of this mechanism, Bottazzo et al. recently reported that immunofluorescent studies on pancreatic frozen sections from a 12-yr-old girl who had recent-onset IDDM (HLA type unknown) showed rare la-positive islet cells that were insulin positive but none that were either glucagon or somatostatin positive. The same group observed similar findings with immunofluorescence on frozen sections of pancreata from newly diabetic BB-Wistar rats. In our study, we examined the effects of LK supernatants and recombinant IFN-γ on la antigen expression in isolated murine whole islets and dispersed islet cells.

METHODS AND MATERIALS

Islets were isolated from male B10.BR/SgSnJ (H-2^k) mice or Balb/c/J (H-2^b) mice (Jackson Laboratories, Bar Harbor, ME) by the collagenase technique and separated on a discontinuous ficoll gradient. Islets were removed from the
was inactivated with tissue culture medium. Single cells T-cell line sensitized to were incubated with either one of several LK supematants fluorescent microscope. Other islets were treated with goat EDTA-versene (1:5000) for 7 days and then stained as above with an anti-IA k samples were prepared as wet mounts and viewed under a 4°C. After three washings, the islets were incubated with 100 samples were collected as wet mounts and viewed under a fluorescent microscope. Negative controls for insulin were normal guinea pig γ-globulin followed by rabbit anti–guinea pig IgG-TRITC and rabbit anti–guinea pig IgG-TRITC alone. An antioxidant [1,4-diazabicyclo(2,2,2)octane; Sigma, St. Louis, MO] was used to slow the quenching of the fluorescent for photography.22

RESULTS
Because whole islets contain several thousand cells and are approximately spherical, only staining of cells on the surface of the islets could be readily evaluated. One to three la-positive dendritic cells per islet were found in islets stained immediately after isolation. These la-positive cells were rarely identifiable after 3 days of culture under standard conditions but could often be identified when cultures were supplemented with 5% LK. No la-positive islet parenchymal cells were seen either immediately after isolation or after 3 days of culture with 5% LK.

After 5 days of culture with 5% LK, many individual islet cells or groups of islet cells were outlined by irregular, granular fluorescent staining (Fig. 1). Neither longer incubation nor 10% LK appreciably increased the number or staining intensity of la-positive islet cells. Similar staining, although less intense, was apparent on islets cultured with 10, 100, or 1000 ng/ml IFN-γ for >6 days. Maximal staining was seen at the two higher concentrations. Staining for la was specific. B10.BR (H-2b) islets stained with the 10-2.16 antibody (anti-IAb) but not the MKD6 antibody (anti-IAd); Balb/c (H-2d) islets stained with the MKD6 antibody but not the 10-2.16 antibody. Islets treated with only goat anti-mouse IgG-FITC were not stained.

Because dispersed islet cells tend to reaggregate during culture, it was not possible to maintain a pure culture of single cells. However, single and small clumps of islet cells from B10.BR mice were stained for la with A.TH anti-A.TL serum. Ten to forty percent of islet cells incubated with 5 or 10% LK expressed la antigens. Maximal la antigen expression was seen after >10 days culture with LK. La-positive islet parenchymal cells were both insulin positive and insulin negative (Fig. 2, A and B).
FIG. 2. Dual immunofluorescence for insulin (A) and la antigen (B) shows 2 insulin-positive β-cells that are la positive (left) and 1 insulin-negative non-β-cell that is la positive (right). Corresponding cells in both micrographs are marked with arrows.

DISCUSSION

Our study demonstrates for the first time that IFN-γ can induce la antigen expression on islet parenchymal cells. Two previous in vitro studies examined the effects of IFN-γ on class II MHC molecule expression in isolated islets. Bottazzo et al. was not able to induce them on either adult rat or 13- to 20-wk human fetal gestation islets incubated with the mitogen phytohemagglutinin; the exact conditions of these experiments were not reported. Campbell et al. studied the effect of 48-h culture with 2 U/ml recombinant murine IFN-γ on dispersed CBA (H-2k) mouse islet cells with a fluorescence-activated cell sorter and various monoclonal antibodies. They found a 10-fold increase in H-2K expression and 2-fold increases in H-2D and la antigen expression. The 2-fold increase in la antigen probably represented enhanced expression on passenger leukocytes, because they were not able to demonstrate any la-positive β-cells with dual immunofluorescence.

Several factors may explain why our results differ from those of Campbell et al. First, the duration of culture with IFN-γ was longer in our study. We did not see la antigen expression on whole islets until the 5th day of culture with LK. When we used dispersed islet cells, even longer culture periods were required. Second, the concentrations of IFN-γ used in our study were at least 60-fold greater. Finally, there is a report that 20% of untreated islets isolated from CAP, DA, and LEW rats contained 20–40% weakly la-positive islet cells. We and others have not been able to identify basal expression of la in nonstimulated mouse or rat islets.

The expression of la by β-cells could have importance in the context of IDDM. It may mean that the β-cell presents its own autoantigenic antigen and therefore initiates autoimmunity. To do this, however, the β-cell would also have to produce the other auxiliary molecules (e.g., interleukin 1) required for T-cell stimulation. Whether the β-cell has this property is not known. la-bearing β-cells also need to be tested directly for antigen presentation in order to place our observations in their correct perspective. Nevertheless, our studies, combined with the in vivo findings of occasional la-positive β-cells, leave little doubt that the β-cell can react as other epithelial cells to produce this protein that is essential for initiating an immune response.

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