Analysis of RNA transcripts for HLA class II genes in human small intestinal biopsies

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SUMMARY Studies of the expression of selected genes within the intestinal mucosa will provide important new information about physiologic and pathological processes that effect mucosal growth, differentiation, and function. To study gene expression in the gut, we developed a method to obtain sufficient undegraded RNA from human endoscopic intestinal biopsy specimens for Northern and slot blot analysis. To verify the method, we examined the differential expression of HLA class II genes in small intestinal mucosa. Levels of RNA transcripts for HLA-DR, -DP, and -DQ \( \alpha \) and \( \beta \) chains were assessed in freshly isolated endoscopic intestinal mucosal biopsy specimens and compared with levels in Epstein-Barr virus transformed B cells from the same individuals. Sufficient undegraded cellular RNA with distinct 28S and 18S ribosomal bands could be obtained from as few as two 2–3 mm endoscopic biopsies. Using chain and locus specific cDNA probes, HLA-DR, -DP, and -DQ subregion genes were shown to be expressed in intestinal mucosa, with the relative magnitude of RNA transcripts being DR>DP>DQ. The same hierarchy of expression was seen for EBV-transformed B cell lines. This method, in conjunction with the polymerase chain reaction for amplifying specific RNA transcripts and in situ hybridisation methods for the cellular localisation of RNA transcripts, will enable studies on the regulation of gene expression in the intestinal mucosa.

The differential expression of genes within the intestinal mucosa determines normal and pathological development and function in the gut. Nonetheless, it has been difficult to study gene regulation in human gut because of difficulty in obtaining sufficient quantities of tissue and a lack of appropriate in vivo or in vitro models.

Human HLA class II genes are located in the HLA-D region of the major histocompatibility complex (MHC) on chromosome 6. The HLA class II genes encode three major sets of HLA class II antigens that are expressed on the cell surface; HLA-DR, -DP, and -DQ. The HLA class II antigens are heterodimers, each consisting of an \( \alpha \) and \( \beta \) chain.\(^5\) HLA class II antigens are found normally on cells of the lymphoid system, including B lymphocytes, macrophages/dendritic cells, and activated T cells.\(^5\) The expression of HLA class II molecules also can be stimulated, particularly during inflammation, on other human non-lymphoid cell types, including epithelial cells – for example, biliary, gastric, small and large intestinal, thyroid epithelial cells, and vascular endothelial cells.\(^6,7\)

The HLA class II antigens are highly polymorphic and, as such, are important in the presentation of a wide array of antigenic peptides to T lymphocytes of the CD4 lineage.\(^8\) Several diseases of a presumed autoimmune nature, including coeliac disease, have a significant association with specific HLA antigens and HLA class II haplotypes.\(^7\) Further, the inappropriate expression of HLA class II antigens, on cells that normally do not express those molecules, has been postulated to be important in the pathogenesis of several autoimmune diseases that have strong HLA class II associations.\(^9,10\)

Antigens coded by HLA-DR subregion genes have been shown on normal villous absorptive epithelial cells of the human small intestine by immunohistochemical and immunoelectronmicroscopic methods.\(^11-14\) Further, patients with coeliac disease, compared with controls, have greater expression of HLA-DR on epithelial cells in the crypt region after a gluten challenge.\(^15-18\) Little is known, however, regarding the mechanism by which HLA-DR, -DP,
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and -DQ molecules are differentially regulated in the gut or the function of HLA class II antigens in the intestinal mucosa.20-23

Immunohistochemistry permits a qualitative analysis of the morphologic distribution of antigens in the intestine. Such approaches are not well suited, however, for investigations of gene regulation and the factors affecting gene expression in the intestine. The latter require several complementary approaches, one of which is the ability to directly detect and quantify specific RNA transcripts within the intestinal mucosa. A serious obstacle to such studies has been the limited amount of small intestinal tissue readily available for study in the absence of surgery, coupled with the problem of rapid degradation of cellular RNA by RNAses in tissue obtained at surgery or post-mortem where there is a delay in tissue processing. Using HLA class II genes as a model, we report herein that sufficient undegraded cellular RNA can be obtained from as few as two intestinal endoscopic biopsy specimens for Northern and slot blot analysis of RNA transcripts in small intestinal mucosa.

Methods

Patients and cell lines

Mucosal biopsies were obtained from the second portion of the duodenum of normal subjects at the time of fibreoptic gastroduodenoscopy. Biopsies were placed immediately in guanidinium isothiocyanate. Concurrently, 30–50 ml of blood was drawn into sodium heparin-containing tubes (15 U/ml), and the peripheral blood lymphocytes were separated by Ficoll-Hypaque gradient centrifugation (Pharmacia, Fine Chemicals, Piscataway, NJ). Lymphoblastoid B-cell lines were initiated as previously described.25 Briefly, 5×10⁶ peripheral blood lymphocytes were cultured in RPMI-1640 media, supplemented with 2 mM glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin and 10% fetal calf serum, to which supernatant from an Epstein-Barr virus shedding cell line, 1437, and 2 µg/ml cyclosporin A were added.

Isolation of RNA and Northern blot hybridisation

RNA preparation and Northern blot analysis were performed as described before,25-28 with the following modifications. To obtain cellular RNA, mucosal biopsies were placed immediately at the bedside in 3 ml guanidinium isothiocyanate containing 2-mercaptoethanol, and then homogenised with a Polytron homogeniser (Brinkmann Instruments, Inc, Orange, CA) on ice for three to 10 seconds. Specimens were then centrifuged for three minutes to minimise contamination with pieces of connective and adipose tissue, after which supernatants were gently forced through a no. 22 gauge needle with a sterile syringe. RNA was prepared from supernatants by the guanidinium isothiocyanate caesium chloride procedure,29 followed by phenol/chloroform extraction.30 For Northern blot analysis,31 RNA samples (5–10 µg) were dissolved in 50% formamide and 2.2 M formaldehyde and electrophoresed in a 2:2 M formaldehyde/1% agarose gel in 20 mM morpholinopropane sulphonic acid buffer. RNA samples were transferred by capillary blotting in 20×SSC (1×SSC = 150 mM NaCl, 15 mM trisodium citrate, pH 6) to Biotrans nylon membranes (ICN Corp, Irvine, CA) according to manufacturer’s instructions. Air dried filters were baked in a vacuum oven for two hours at 80°C and prehybridised for four to six hours at 42°C in 50% formamide, 5×SSC, 50 mM NaPO₄ pH 6.5, 0-1% SDS, 250 µg/ml sonicated salmon sperm DNA, and 5 µg/ml polyuridylic acid (Pharmacia Co, Piscataway, NJ). Hybridisation was performed for 12–16 h at 42°C in fresh prehybridisation solution with the addition of 10% dextran sulfate and radiolabelled probe (10⁶ cpm/ml). ³²P radiolabelled cDNA probes were prepared by the random priming method of Feinberg et al.32 using high specific activity α-³²P dCTP (3000 Ci/mmol). Final washes (2×) were done at a stringency of 0.1×SSC at 60°C for 30 min each. Nylon filters were exposed to Kodak XAR-5 x-ray film with an intensifying screen for 72 h. For rehybridisation with different probes, blots were stripped in 50% formamide, 5 mM Tris-hydrochloric acid (pH 7-0) and 1 mM EDTA, at 65°C for two 30 min periods. Each blot could be rehybridised five to eight times. Slot blots on nitrocellulose paper were treated similarly after two-fold serial dilutions of RNA.

HLA class II gene cDNA probes

HLA-DR, -DQ, and -DP α and β chains and locus specific cDNA’s were kindly provided by Dr Piatier-Tonneau.33 Hybridisation probes used were as follows: a 460 bp Pst I fragment of LB 24 cDNA, a DP α chain cDNA; a 500 bp Rsa I-Sac I fragment of pDCH1 cDNA, a DQ α chain cDNA; a 700 bp Pst I-Pst I fragment of pDRH-7, a DR α chain cDNA; a 130 bp Pst I-Rsa I fragment from the 3’ UT region of pDR β, a DR β1 chain cDNA; a 497 bp Pst I fragment of the 3’ UT region of the DC3 β gene, a DQ β genomic clone; a 370 bp Sac I-Hinc II fragment of the 3’ UT region of DP4 β, a DP β genomic clone and a 300 bp Ava II-Hind III fragment of the 3’ UT region of PMT3 β-3 (MT), a DR β3 genomic clone.34 A human α tubulin cDNA, was provided by Dr N J Cowan.35 Hybridisation probes were tested and shown to be chain and locus specific by hybridisation of the inserts with ³²P nick-translated whole plasmid.
Results

We obtained a yield of approximately 40 μg of total RNA from two 2–3 mm endoscopic biopsy specimens, sufficient for Northern and slot blot analysis. The pattern of undegraded cellular RNA with distinct 28S and 18S ribosomal RNA bands is shown in Figure 1. A representative Northern blot, using RNA from intestinal biopsies or EBV-transformed B cell lines from the same individuals and hybridised with HLA class II α chain probes, is shown in Figure 2. Intestinal biopsies and the EBV B cell lines expressed high levels of DR-α transcripts. Similarly, DP-α transcripts were highly expressed in intestinal specimens and EBV cell lines. RNA transcripts for DQ-α were expressed at a lower level both in the intestinal biopsies and EBV cell lines. Parallel results were obtained for HLA-DRβ, DPβ, and DQβ chains (not shown). Among the HLA class II RNA transcripts, three different patterns were observed; a single RNA band was seen for DR- and DP-α (1.5 kb) (Fig. 2) and DR-β and DP-β (not shown). Two bands were seen for DQ-β (1.3 kb and 1.7 kb) (not shown) and three bands were seen for DQ-α (1.3, 1.5, and 1.7 kb) (Fig. 2). Polymorphism in the size of DQ-α RNA transcripts is known to correlate with the
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Fig. 3  Slot blot analysis of RNA from intestinal biopsies of three different individuals probed with HLA-DR, -DP, or -DQα chain cDNAs. Slots contain serial two-fold dilutions of RNA. Hybridisation signals are DR>DP>DQ.

The reproducible success of this procedure was at first surprising, given the problems we anticipated from RNA degradation by cellular RNAses in that site. In fact, our initial attempts to obtain RNA using a single step extraction method were not successful. Our success with the method reported herein appears to depend on the rapid transfer of biopsy specimens to guanidinium isothiocyanate, the use of small volumes, and care to avoid contaminating RNAses during harvesting of samples from the CsCl gradients.

To verify the utility of our method, we examined HLA Class II gene expression in intestinal mucosa as assessed by the level of mRNA transcripts for HLA-DR, DP, and DQα and β chains. The present report demonstrates that HLA-DR, -DP, and -DQ sub-region genes are expressed by cells within the normal intestinal mucosa. Quantitative analysis of slot blots revealed that the relative order of the steady state mRNA levels are DR>DP>DQ. Previously, it has been controversial as to whether HLA-DR and -DQ class II antigens are expressed by cells in this site in the absence of disease.

We note that the contribution to the expression of HLA class II RNA transcripts by absorptive epithelial cells compared with B lymphocytes and other mononuclear cells in the lamina propria was not being assessed by this approach. In contrast with genes that are expressed selectively in defined cell types - for example, epithelial cells, goblet cells, enteroendocrine cells, molecules like those encoded by HLA genes are expressed by multiple cell types in the intestinal mucosa. For genes expressed by multiple cell types, the quantitative measurement of RNA transcripts in extracts of whole mucosa, as described herein, can be complemented by in situ hybridisation studies to document the cell type(s) producing the RNA transcripts being studied. In situ hybridisation, however, simply provides a qualitative picture of the cells that are expressing a particular gene. Filter hybridisation methods, as presented here, are required to analyse the relative levels of specific transcripts in biopsies from the normal intestinal mucosa and alterations in gene expression in different disease states. Finally, we note that total cellular RNA, as prepared herein, can serve as a template for the synthesis of single strand cDNA, which subsequently can be used to amplify specific transcripts by the polymerase chain reaction.

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