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INDUCTION OF THE INSULIN RECEPTOR AND OTHER DIFFERENTIATION MARKERS BY SODIUM BUTYRATE IN THE BURKITT LYMPHOMA CELL, RAJI

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The very low expression of insulin receptors in the Burkitt lymphoma cell Raji was increased 2-fold, 6-fold and 10-fold after 1,2 and 3 days, respectively, by incubation with the differentiation inducer sodium butyrate. Insulin receptor number was increased without a change in receptor affinity, in association with an increase in the receptor α and β subunits detected after cell-surface labelling and immunoprecipitation. Expression of cell-surface class I and II human leukocyte antigens, the intercellular adhesion molecule-1 and the CD38 leukocyte antigen was also increased, consistent with B cell differentiation. Butyrate effects were not unspecific, as the binding of tumour necrosis factor and growth hormone and the expression of the B cell markers CD20, B5 and CD21 was not increased. The low expression of insulin receptors on Raji cells is therefore a reflection of the less differentiated state of these cells compared to lymphoblastoid cells.

We have previously demonstrated that several of Burkitt lymphoma cell lines have a >90% reduction in insulin receptor expression compared to lymphoblastoid cells (1,2). Burkitt lymphoma cells also lack expression of certain activation markers compared to lymphoblastoid cells (3-7) and are therefore considered to be "frozen" at an earlier stage of B cell differentiation. As the insulin receptor is only expressed after activation of resting B cells (8) its reduced expression could reflect the resting B cell phenotype of Burkitt lymphoma cells.

N-butyrate, an aliphatic carboxylic acid, has been shown to induce a variety of morphological and functional changes in different cell types (9-11). Butyrate induces the Burkitt lymphoma cell Raji, classified as a "non-producer" of EBV, to differentiate towards

Abbreviations : BSA, bovine serum albumin; EBV, Epstein-Barr virus; EDTA; ethylenediamine tetraacetic acid; FITC, fluorescein isothiocyanate; HLA, human leukocyte antigen; ICAM-1, intercellular adhesion molecule; M_r, molecular weight; PBS, phosphate buffered saline.

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plasmablast or plasma cell morphology, as well as causing a small increase in EBV antigen synthesis (4). In contrast, the Daudi cell, which is an EBV "producer" and shows marked stimulation of EBV antigen synthesis by butyrate, and the Ramos cell, which lacks EBV expression, are not induced to undergo morphological differentiation (2). We have therefore investigated whether the expression of the insulin receptor increases concomitantly with other markers in Raji cells undergoing butyrate-induced differentiation.

MATERIALS AND METHODS

Materials

[^{125}I]-labelled human insulin (120-140 μCi/μg), human growth hormone (20-30 μCi/μg) and human tumour necrosis factor-alpha (30-40 μCi/μg) were prepared by a modification of the chloramine T method (12). Na[^{125}I] was from Amersham International Ltd. UK.

Monoclonal antibody (W6/32) to class I HLA was obtained from Serotec Ltd., Oxford, England. Other monoclonals were B1 (CD20), B5, WEHI-B2 (CD21), FMC 63 (CD19, the EBV receptor), 2.06 (class II HLA-DR), OKT10 (CD38), OKM-1 (CD11b/CR3), WEHI-CAM-1 (ICAM-1), 5.A10 (insulin receptor) and two control antibodies (anti-Salmonella) AG11 and AG12. Fluorescein-conjugated anti-mouse Ig ($F(ab')_2$ fragments) antiserum was obtained from Silenus/ICI, Melbourne, Australia.

Cell culture

Raji, Daudi and Ramos cells were grown in continuous culture at 37°C in RPMI-1640 medium containing 10% heat-inactivated fetal calf serum. After reaching stationary phase, cells were resuspended in fresh medium at 5 x 10^5 cells/ml. N-butyrate was added to a final concentration of 3 mM. This concentration inhibited cell proliferation but did not significantly affect cell viability over 2 days.

Binding studies

Cells were washed twice with phosphate-buffered saline and resuspended in lymphocyte binding buffer (50 mM Hepes, 120 mM NaCl, 1 mM $MgSO_4$, 1 mM EDTA, 10 mM glucose, 15 mM sodium acetate, 1% BSA, 45 U/ml bacitracin, pH 7.6). Insulin binding to 2 x 10^6 cells was measured at steady state (90 minutes, 15°C) using a fixed tracer amount of ^{125}I-insulin (50,000 cpm, 0.2 ng/ml insulin) in the presence and absence of 20 μg/ml unlabelled insulin, or in the presence of increasing amounts of unlabelled insulin for Scatchard analysis. Cells were washed once with ice-cold lymphocyte binding buffer and cell pellets counted in a 1260 LKB Multigamma counter. Growth hormone binding was carried out in a similar manner except that incubation was for 90 minutes at 30°C with 0.5 ng/ml [^{125}I]-growth hormone in the presence or absence of 1 μg/ml unlabelled growth hormone. Tumour necrosis factor binding was performed for 4 hours at 4°C with 0.5 ng/ml [^{125}I]-tumour necrosis factor in the presence or absence of 5 μg/ml unlabelled tumour necrosis factor.

Cell surface labelling

Cells were washed three times in phosphate-buffered saline containing 20 mM glucose, resuspended in PBS/20 mM glucose (5 x 10^7 cells/ml) and incubated with Na[^{125}I] (1 mCi), lactoperoxidase (150 μg/ml) and glucose oxidase (1 U/ml) for 10 minutes at room temperature. Cells were washed three times with PBS/1 mM KI and solubilized in 0.1 M sodium phosphate pH 7.4, 1% Triton X-100, 2 mM phenylmethylsulfonyl fluoride, 2 mM EDTA, 1000 U/ml aprotinin, 100 U/ml bacitracin for 60 minutes at 4°C. After removing insoluble material by centrifugation,

solubilized receptors were partially purified using wheatgerm lectin-agarose, precipitated using either control or antireceptor antibody and analyzed on reducing 7.5% acrylamide SDS-PAGE gels, as previously described (1).

Cytofluorimetric analysis

Cell surface markers were detected by indirect immunofluorescence. Cells (10^6) were incubated with monoclonal antibody at 4°C for 30 minutes, washed once, incubated with FITC-sheep anti-Ig antibodies for 20 minutes at 4°C, washed three times and analyzed on an EPICS I (Coulter Immunology, Hialeah, FL, USA) cell sorter.

RESULTS AND DISCUSSION

Insulin binding increased 2-fold after 1 day ($p<0.05$), 6-fold after 2 days ($p<0.01$) and 10-fold after 3 days ($p<0.001$), when Raji cells were cultured with 3 mM sodium butyrate (Fig. 1). Competition binding studies and Scatchard analysis revealed that the increase in binding was due to an increase in the number of insulin receptors while the apparent affinity of the receptor was not altered (Fig. 2). Butyrate-treated cells had similar numbers of insulin receptors as B-lymphoblastoid cells (2). Interestingly, Spira and co-workers (13) showed that EBV transformation of the EBV-negative Ramos cell resulted in 7-fold increase in insulin receptor expression. When Raji cells were cultured for 2 days in the presence of butyrate and [^{125}I]-surface-labelled, solubilized and desorbed from wheatgerm lectin-agarose, precipitated with anti-receptor antibody and analyzed by SDS-PAGE, there was an increase in the insulin receptor α (M_r 130,000) and β (M_r 90,000) subunits (Fig. 3). A M_r 210,000 protein was also detected, identical in size to the uncleaved receptor precursor identified by biosynthetic

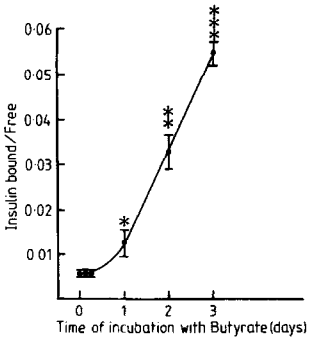

Figure 1 : **Time course of insulin receptor induction in Raji cells by butyrate.** Cells were incubated for varying times in the presence of 3 mM sodium butyrate and insulin binding measured as described in the Methods. Results are expressed as the mean of 3 experiments ± S.E.M. Differences compared to time zero were tested by Student's T test (*$p<0.05$, ** $p<0.01$, *** $p<0.001$)

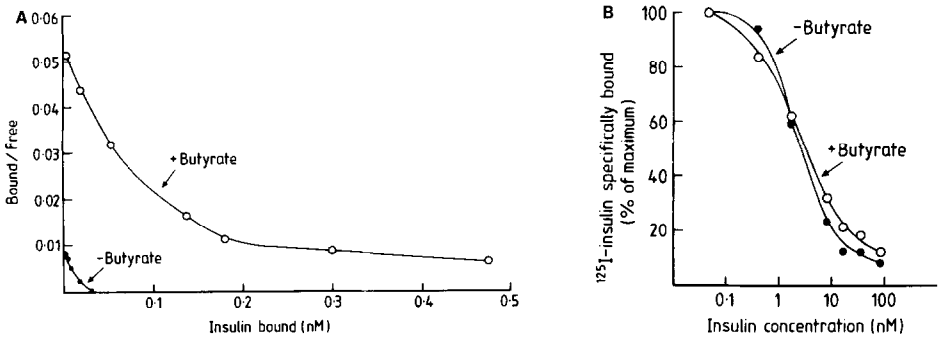

Figure 2 : Scatchard plot (panel A) and competitive inhibition plot (panel B) of insulin binding to Raji cells cultured for 3 days in the presence or absence of 3 mM sodium butyrate. Insulin binding was measured as described in Materials and Methods.

labelling (14). Only minor amounts of this M_r 210,000 protein are detected in B-lymphoblastoid cells, although in the Burkitt lymphoma cells Raji and Daudi it is about as abundant as the M_r 130,000 α subunit, possibly reflecting lower turnover of receptor in these cells (1). Consistent with this possibility, butyrate treatment of Raji cells

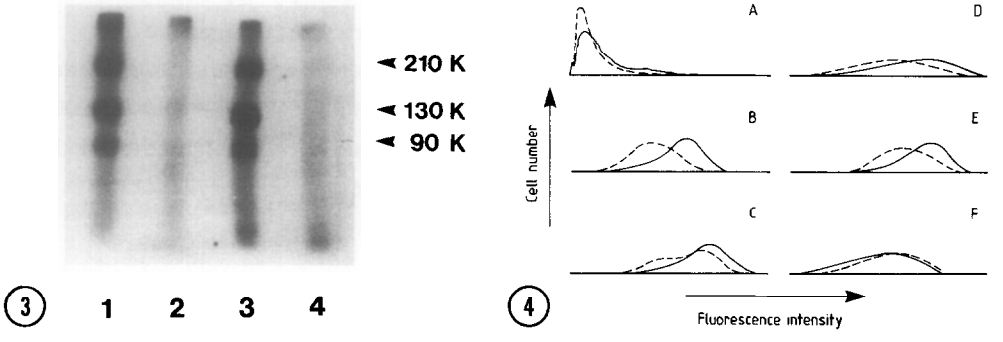

Figure 3 : Cell surface labelling of insulin receptors on Raji cells. Raji cells were cultured for 2 days in the absence (lanes 1,2) and presence (lanes 3,4) of 3 mM butyrate, surface-labelled with Na[^{125}I], solubilized, partially purified by desorption from wheat germ lectin-agarose, precipitated with either antireceptor antibody (lanes 1 and 3) or control antibody (lanes 2 and 4) and analyzed by SDS-PAGE using 7.5% SDS-acrylamide gels.

Figure 4 : Fluorescence intensity profiles of antibody-labelled markers on Raji cells. Cells were cultured for 2 days in the presence (____) or absence (---) of 3 mM sodium butyrate before analysis of cell surface markers by indirect fluorescence.

Panel A - AG11 control antibody (anti-Salmonella)
Panel B - insulin receptor (5.A10)
Panel C - CD38 (OKT10)
Panel D - class II HLA-DR (2.06)
Panel E - ICAM-1 (WEHI-CAM-1)
Panel F - CD19 (FMC 63)

TABLE 1 : INSULIN BINDING TO THE BURKITT LYMPHOMA CELLS, DAUDI AND RAMOS

	Specific Bound/Free [^{125}I] Insulin
Daudi	0.005
Daudi + butyrate	0.007
Ramos	0.017
Ramos + butyrate	0.016

Cells were cultured for 2 days in the absence or presence of 3 mM sodium butyrate. Insulin binding to 2 x 10^6 cells was measured as described in Materials and Methods.

resulted in a decrease in the the M_r 210,000 protein relative to the M_r 130,000 subunit.

Cytofluorimetric analysis confirmed the increase in insulin receptor expression in butyrate-treated cells (Fig. 4). Increases in CD38, class II HLA-DR and ICAM-1 expression and a slight decrease in CD19 expression were also found in butyrate-treated cells (Fig. 4). These changes are consistent with B cell differentiation (15). There was also a slight increase in the expression of CD11b/CR3, but no change in the CD20, B5 or CD21 (EBV receptor) (results not shown). Specific binding of [^{125}I]-labelled growth hormone or tumour necrosis factor to Raji cells was $\leq$1% of total tracer hormone and was unchanged by butyrate treatment (results not shown). The effect of butyrate to increase insulin receptor expression on the Raji cell was therefore selective. Butyrate had no effect (Table 1) on insulin binding to the two other Burkitt lymphoma cell lines, Daudi and Ramos, in which it fails to induce morphological differentiation.

Thus, the low expression of insulin receptors on the Raji cell and, by inference, some other Burkitt lymphoma cells (1,2), most likely reflects the early stage of differentiation, ie. the resting B cell phenotype, of these cells. Our findings suggest that the insulin receptor is a marker of B cell differentiation and confirm that the phenotype of the Raji cell is not "frozen" and can be converted to one characteristic of lymphoblastoid cells.

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