Erythrocyte and Monocyte Insulin Binding in Man

A Comparative Analysis in Normal and Disease States

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SUMMARY

Blood cells have been widely used to evaluate the status of the insulin receptor in man. The insulin receptor of human monocytes has been shown to mirror pathophysiologic states of insulin receptors in target tissues of animal models. Thus, comparison of the properties of the insulin receptor studied simultaneously in erythrocytes and monocytes is appropriate. We studied 19 normal subjects and 32 patients with diverse conditions such as acromegaly, insulinomas, insulin-dependent diabetes mellitus, corticosteroid administration, and the Type A and B forms of insulin resistance. We find that specific insulin binding at tracer concentrations significantly correlate ($P < 0.001$) for the two cell types obtained from these individuals. However, the total number of binding sites (R_0) and affinity of the receptor at the lowest insulin concentration ($\bar{K}_e$) do not significantly correlate ($P > 0.1$). Monocytes from patients with the Type A and B forms of insulin resistance exhibit marked alterations in insulin binding and simultaneous studies in erythrocytes reflect these changes. The administration of 40 mg/day of prednisone for 3 days to normal subjects produced no significant change in insulin binding to either cell type. Insulin binding to both cell types from poorly controlled insulin-dependent diabetes mellitus patients was similarly normal or elevated. However, improved control decreased insulin binding in monocytes, but not in erythrocytes. We conclude that there is a general relationship between monocyte and erythrocyte insulin binding, but important differences exist in the way their insulin receptors are regulated. Insulin binding in erythrocytes is inversely and exponentially related to cell age. Unlike erythrocytes, monocytes represent a more uniform population of cells capable of the same receptor-mediated endocytotic functions as hepatocytes. Thus, the monocyte may provide a clearer reflection of the insulin receptor status in target tissues. DIABETES *30*:896–902, November 1981.

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Circulating blood cells have been used extensively for evaluating the insulin receptor status of human subjects. In 1972, Gavin et al.[1] first demonstrated insulin binding to mononuclear cells, granulocytes, cultured human lymphocytes, and erythrocytes. After the demonstration that the monocyte is the predominant insulin binding cell of blood,[2] these cells have been used most extensively for human studies. More recently, Gambhir et al.[3] introduced improved techniques for the binding of insulin to erythrocytes. This development has led to more extensive use of these cells for human studies.

Heterogeneity in mononuclear cell populations is not presently known to affect insulin binding, as long as a correction is made for the percentage of monocytes. However, heterogeneity in erythrocyte populations clearly may affect insulin binding. Different age groups of red cells from a single individual have distinctly different degrees of insulin binding, and hematologic disorders that alter the normal 60-day mean cell age of whole blood may significantly affect binding.[4] We have shown that insulin binding to human erythrocytes is not only inversely,[5] but also exponentially related to their mean cell age.[6]

In our laboratory, monocytes have been used extensively to study insulin binding in patients. More recently, simultaneous red cell and monocyte studies have been carried out in patients with a variety of clinical conditions where insulin binding varies over a wide range. These include normal volunteers, acromegalic patients,[7] patients with insulin-secreting tumors,[8] with insulin-dependent diabetes mellitus in different states of metabolic control,[9] with short-term corticosteroid treatment,[10] and patients with acanthosis nigricans and insulin resistance.[11] In this paper, we report the results of erythrocyte insulin receptor studies and compare these to simultaneous studies of monocyte insulin receptors.

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MATERIALS AND METHODS

^{125}I-labeled porcine insulin was prepared.[12] Insulin binding to red cells and monocytes was performed as previously described.[13] Red cells were separated from mononuclear cells using two replicate Ficoll-Hypaque gradients. After each gradient, the top-most layer of red cells, usually less than 5% of the original volume of erythrocytes, was aspirated along with the polymorphonuclear cell layer and discarded.[3] The percent specific ^{125}I-insulin bound was calculated from cell samples incubated in the absence and presence of insulin concentrations of 10^5 ng/ml. Competition curves and Scatchard plots were constructed and analyzed to derive the parameters of nanograms of insulin required for 50% maximal displacement of ^{125}I-insulin (ID_{50}), maximum insulin binding capacity (R_o), and affinity of the empty receptor ($\bar{K}_e$).[14] Methods used to derive these parameters are illustrated in Figure 3.

Samples of blood (100 ml) blood were taken for assay in the morning from both patients and volunteers at the Clinical Center of the National Institutes of Health, after an overnight fast. Buffy coat specimens of blood were obtained from seven normal volunteers by plasmapheresis. These specimens were prepared by centrifugation of one unit of blood at 1600 × *g* or 600 × *g* for 3 or 9 min, respectively, whereupon the top layer of cells (30–80 ml) was collected. The remainder of the blood was then either returned to the volunteer or donated to the blood bank. A total of 32 patients and 19 normal volunteers participated in these studies.

Differences between the data from independent groups of subjects were evaluated using Student's unpaired *t* test. Differences between paired data were evaluated using Student's paired *t* test.

RESULTS

Effect of sample collection and processing on insulin binding. In general, samples have been obtained for blood cell insulin binding studies either from the buffy coat of a 450-ml unit of blood or directly from a 100-ml aliquot of whole blood. To determine whether the method of collecting the sample affects insulin binding, plasmapheresis samples were compared with the standard 100-ml samples. Erythrocyte insulin binding was consistently greater in the plasmapheresis samples as compared with the standard samples (Figure 1). This effect results from increased concentration of younger red cells with lesser buoyant density in the buffy coat. By contrast, no significant difference in monocyte insulin binding was observed in samples obtained by the two methods (Figure 1). Parenthetically, it should be mentioned that only a 10-ml blood sample is required for erythrocyte insulin binding. Different methods such as centrifugation through Ficoll-Hypaque and aspiration of the top layer of the specimen or passage through a cellulose column[15] may be used to free these samples of leukocytes. We have not fully evaluated these different methods with respect to their effect on erythrocyte insulin binding. In this study, larger quantities of blood were required for the combined erythrocyte and monocyte assays reported.

Comparison of erythrocyte and monocyte insulin binding in patients and normal volunteers. Insulin binding to erythrocytes and monocytes was studied in 50 samples of blood from 13 normal volunteers and 32 patients over a 2-yr

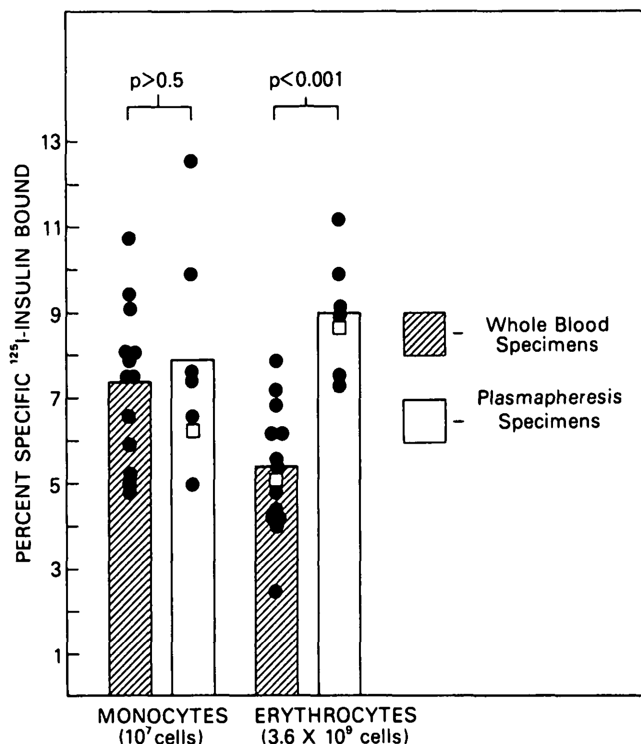
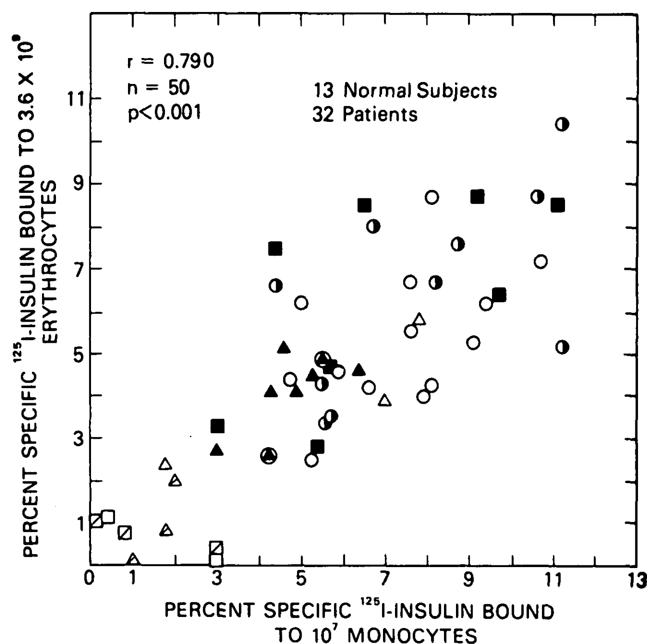


FIGURE 1. Erythrocyte and monocyte insulin binding in 19 normal volunteers. Seven plasmapheresis specimens and 14 whole blood specimens were studied. Plasmapheresis and whole blood specimens were analyzed on the same day in only one subject (□) as shown. Separate samples (1 plasmapheresis and 13 whole blood specimens) from 13 subjects were assayed at different times. Insulin binding data from these 13 whole blood specimens is shown in Figure 3. Based on these studies, data from patients whose erythrocytes were obtained by plasmapheresis were excluded from Figures 2 and 7 since such samples contain an increased concentration of younger red cells and were presumed to have higher insulin binding than whole blood.

FIGURE 2. Percent specific ^{125}I-insulin bound to 50 samples of erythrocytes and monocytes. Normal subjects (○), N = 13; poorly controlled insulin-dependent diabetics (◑), N = 10; patients with an insulin secreting islet cell tumor (■), N = 8; acromegalic patients (▲, Ⓐ), N = 8; Type A patients (△, ◬), N = 6; Type B patients (□, ▨), N = 6. The symbols Ⓐ, ◬, and ▨ designate replicate studies in individual patients.



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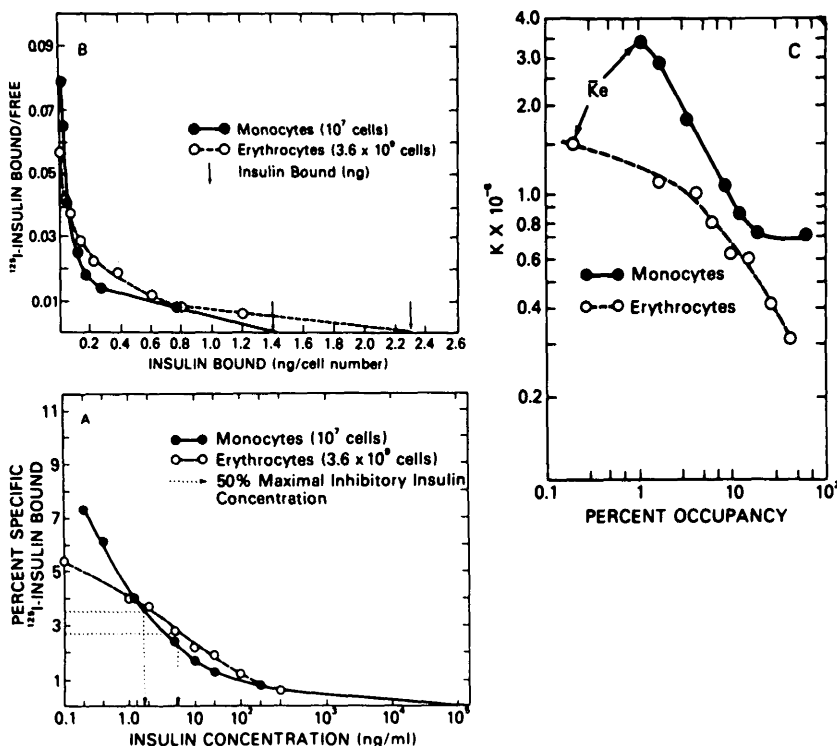

FIGURE 3. Insulin binding in normal subjects (10 females and 3 males). (A) Insulin binding competition curves and determination of ID_{50}. (B) Scatchard plots and determination of R_o. As the lower limiting B/F is reached, each point becomes much less precise. We have found that the R_o (the B at saturation) is more accurately estimated from an extrapolation as shown above. This has been the method used to derive R_o from monocyte binding curves in all studies previously reported from this laboratory. Because the shape of the competition curve from the two cell types is slightly different, extrapolation was made from the 200-ng/ml point as shown for erythrocytes rather than the 100-ng/ml point as shown for monocytes. While this method is arbitrary, it can be used to compare different experimental groups and correlates well with a computer fit of the data (R. S. Bar, personal communication). This method of data analysis should be appropriate for linear correlations, and correlations will not be improved by other methods of deriving R_o. (C) Affinity profiles and determination of $\bar{K}_e$. Monocyte ●—● and erythrocyte ○—○ insulin binding data from 13 blood samples.

period. The data are expressed in four different ways. First, the percent specific ^{125}I-insulin bound to the two cell types was compared and a highly significant correlation was found ($r = 0.79$, $P < 0.001$) (Figure 2). However, within each subgroup or class of patients or in the normal group (Figure 2), no significant relationship ($P > 0.05$) between percent specific ^{125}I-insulin bound to the two cell types could be demonstrated.

Next, competition curves were carried out and analyzed for all subjects. Typical competition curves, Scatchard plots, and affinity profiles as determined for the mean data obtained from each cell type from normal individuals are shown (Figure 3). In 42 samples, the insulin concentration required for displacement of the half-maximal ^{125}I-insulin bound (ID_{50}) could be calculated. These parameters correlated to a lesser, but still significant degree ($r = 0.36$, $P < 0.05$) (Figure 4A).

Since derived parameters of insulin binding (R_o and $\bar{K}_e$)

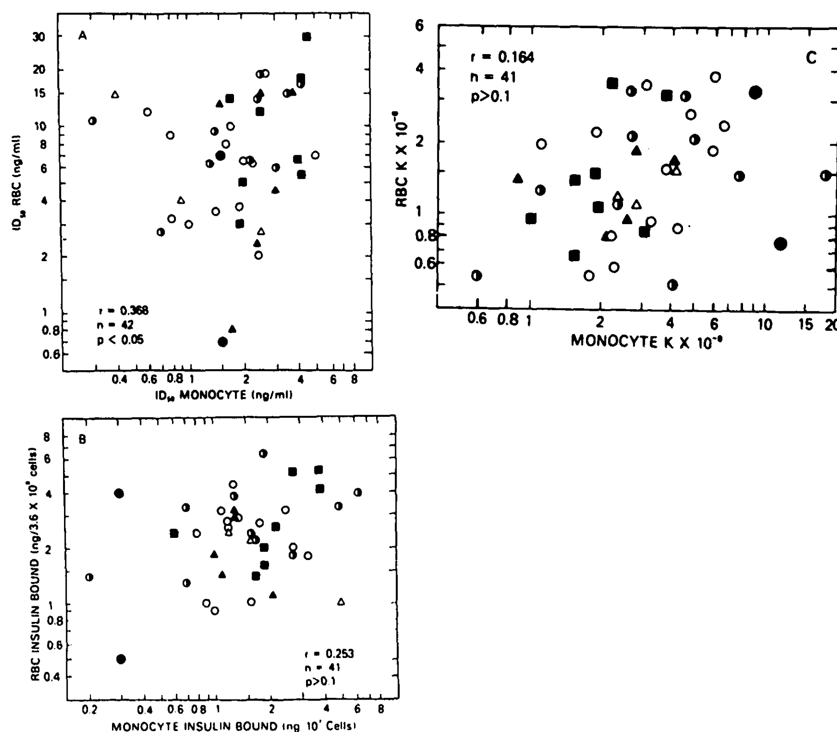

FIGURE 4. Comparison of derived parameters of insulin binding in monocytes and erythrocytes. (A) Insulin concentration for 50% displacement of ^{125}I-insulin (ID_{50}). (B) Maximal insulin binding capacity (R_o). (C) Affinity of the insulin receptor ($\bar{K}_e$). Data from all Type B patients and one Type A patient was excluded from this figure (see results). One acromegalic patient was excluded from B and C because there were insufficient numbers of monocytes for complete analysis.

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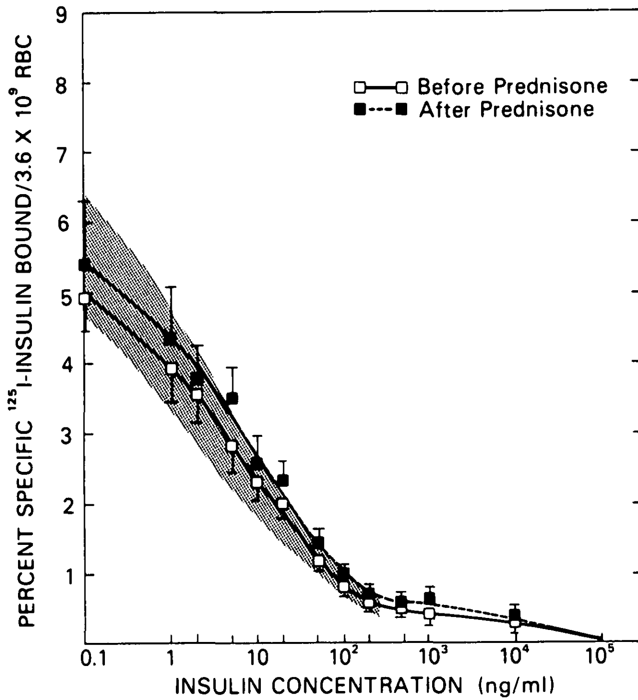

FIGURE 5. Insulin binding to erythrocytes before (□—□) and after (■---■) three days of oral prednisone (40 mg/day given to 9 normal females). Shaded area is ±2 SEM for data from 13 normal individuals. Error bars show ±1 SEM.

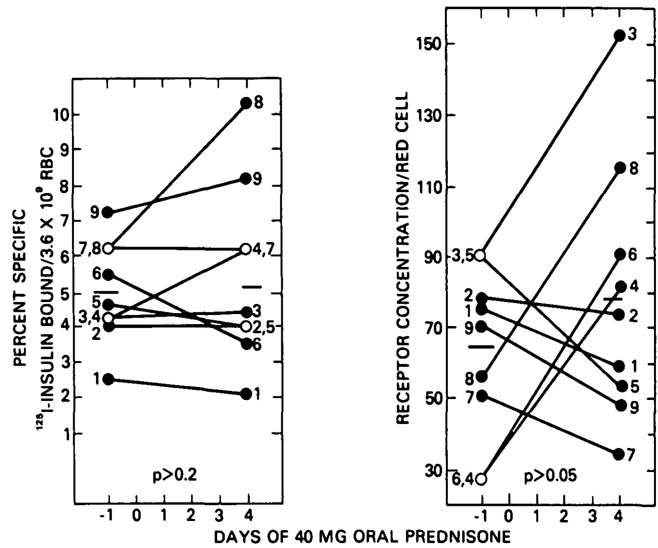

FIGURE 6. Effect of prednisone on percent specific ^{125}I-insulin bound and receptor concentration/red cell in nine individual female subjects. Receptor concentration was calculated as described in reference 13.

have been used extensively to characterize insulin binding capacity and the affinity of the receptor for insulin,[14] these parameters were calculated and correlated (Figure 4B and 4C). In 41 samples, no significant correlation was found for either R_0 or $\bar{K}_e$ in the different cell types. In this study we excluded some Type A and B patients because the very low insulin binding to the cells from these subjects precluded derivation of R_0 and, hence, $\bar{K}_e$. Note, however, that when such subjects are excluded from Figure 2, the correlation in percent specific ^{125}I-insulin bound is still highly significant ($r = 0.63$, $P < 0.001$, $N = 42$).

Effect of prednisone and improved diabetic control on insulin binding. We next evaluated two different situations in which the insulin binding in an individual subject was compared on two or more occasions. In the first of these paired studies, nine normal female volunteers were given 40 mg of prednisone orally for 3 days. Insulin binding to red cells and monocytes was studied before and on the day after treatment. Monocyte data from these subjects has previously been reported.[10] There was no difference in insulin binding to monocytes[10] or erythrocytes (Figure 5) before and after treatment. Examination of both monocyte[10] and erythrocyte (Figure 6) binding data from individual subjects in this group revealed no consistent change in either percent specific ^{125}I-insulin bound or receptor concentration per cell. When the changes in insulin binding to red cells and monocytes from the same individuals in this study were compared, these parameters changed in tandem in both cell types from only two subjects (Table 1).

In the second paired study, six poorly controlled insulin-dependent diabetics had insulin binding studies before and after improved metabolic control. Clinical characteristics and monocyte binding of these patients have been reported previously.[9] Simultaneous erythrocyte and monocyte stud-

TABLE 1*
Change in maximal percent specific ^{125}I-insulin bound (%sB/T) and insulin binding capacity (R_0) in erythrocytes and monocytes after prednisone treatment

Monocyte subject no.	RBC subject no.	RBC (%sB/T)	Monocyte (%sB/T)	Direction of change	RBC R_0	Monocyte R_0	Direction of change
8	1	NC	NC	+	↓	↑	−
4	2	NC	↓	−	NC	↓	−
3	3	NC	↓	−	↑	↑	+
6	4	↑	↑	+	↑	↑	+
7	5	NC	NC	+	↓	↑	−
5	6	↓	↑	−	↓	↓	+
2	7	NC	↓	−	↓	↑	−
9	8	↑	↑	+	↑	NC	−
1	9	↑	↓	−	↓	↓	+
9 total	9 total	5NC 3↑ 1↓	2NC 4↓ 3↑	4+ 5−	1NC 4↓ 4↑	1NC 2↓ 6↑	4+ 5−

* Subject numbers in monocyte studies are from reference 10 by permission. Subject numbers in erythrocyte studies are the same as shown in Figure 6. Direction of change in maximal percent specific ^{125}I-insulin bound and insulin binding capacity of monocytes are from Figure 6 in reference 10. Increase (↑), decrease (↓), no change (NC), with treatment and change in the same (+) or different (−) directions in the two cell types are noted for the nine individual female subjects.

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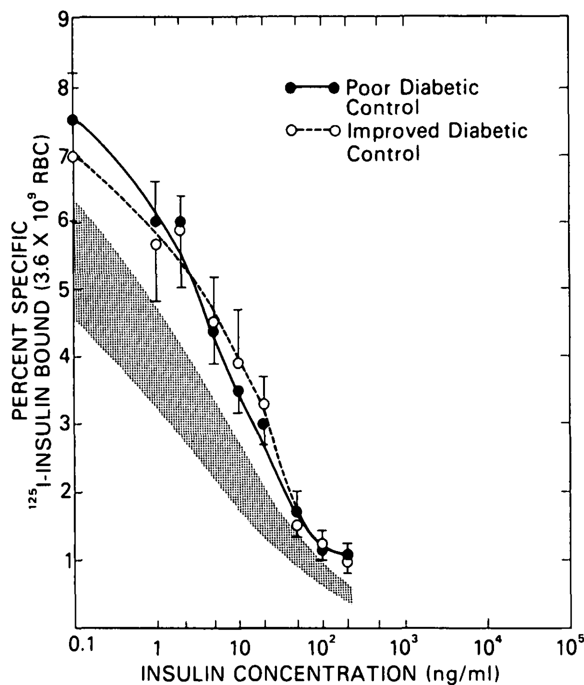

FIGURE 7. Insulin binding to erythrocytes before ●—● and after ○—○ improved diabetic control in five insulin-dependent diabetics (subject no.'s 1, 2, 3, 4, and 8 as reported in reference 9). Shaded area is ±2 SEM for data from 13 normal individuals.

ies were completed before and after improved control in five of the six reported patients. Erythrocyte and monocyte insulin binding was slightly, but not significantly, higher than normal in the patients with poor diabetic control. Insulin binding to monocytes was reduced with improved metabolic control.[9] By contrast there was no significant change in erythrocyte binding under these same conditions ($P > 0.05$) (Figure 7).

Insulin binding to patients with syndromes of extreme insulin resistance. We next wished to compare in detail the monocyte and erythrocyte insulin binding in two forms of severe insulin resistance.[11] The first form has been designated the Type A syndrome. The phenotype of a Type A patient is characterized by insulin resistance, hyperinsulinemia, polycystic ovaries, hirsutism, and variable degrees of glucose tolerance. Insulin binding to monocytes may be either low[11] or normal[16] in these patients. A second form of insulin resistance, the Type B syndrome, is characterized by the presence of autoimmunity with an anti-insulin receptor antibody.[11] Figure 8 shows typical competition curves for erythrocytes from two Type A patients and from a Type B patient. In Type A patients with either normal or low binding to monocytes, insulin binding to erythrocytes was similar. All Type B patients had very low insulin binding to both erythrocytes and monocytes (see refs. 11 and 16 for comparative studies in monocytes).

DISCUSSION

Both circulating monocytes and erythrocytes exhibit insulin receptors. These receptors are similar in their specificity for insulins of different potency, their extractability by detergents, pH optimum, and ability to be inhibited by autoantibodies against the insulin receptor. The most remarkable difference between these two cell types is the age dependence of the insulin receptor on erythrocytes. Clearly, major perturbations in the mean cell age of an erythrocyte preparation will have an effect on insulin binding. These effects can be magnified even by the centrifugation techniques used to isolate red cells.

In the present study, selected adult patients without significant, detectable hematologic disorders were studied. Thus, none of the samples assayed were known to have significant alterations from normal erythrocyte mean cell age. Therefore, we were able to compare insulin binding in simultaneously collected samples of erythrocytes and monocytes in these groups.

Several generalizations appear warranted from these studies and other presently available information. (1) There is a good correlation between the percent specific ^{125}I-insulin bound to red cells and to monocytes taken from large numbers of individuals whose insulin receptors are exposed to a wide variety of conditions and whose metabolic status has not been acutely perturbed. However, results from a smaller number of subjects may not demonstrate this correlation. (2) There is a lesser, but still significant relationship between the shape of the competition curves for the two cell types as exhibited by the amount of insulin needed to displace 50% of the maximal ^{125}I-insulin bound (ID_{50}). (3) Parameters requiring a greater degree of derivation, such as those from Scatchard plots and affinity profiles, do not

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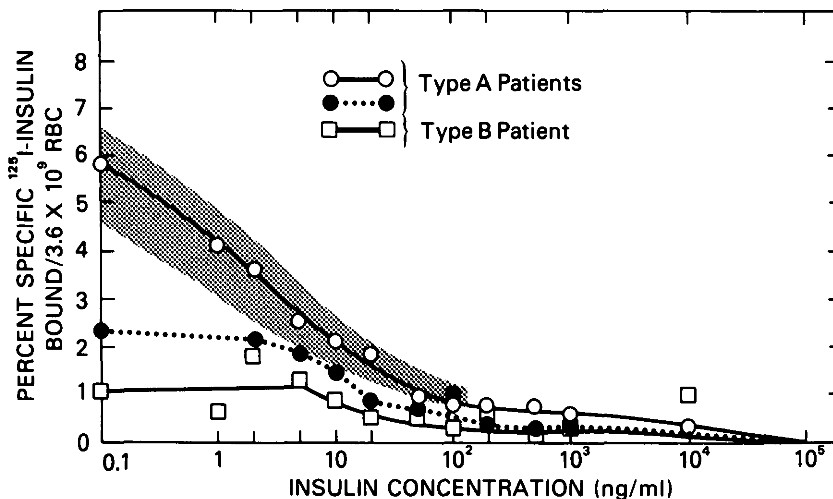

FIGURE 8. Insulin binding to erythrocytes from patients with syndromes of extreme insulin resistance. Patient with the Type A phenotype and normal monocyte insulin binding ○—○ (see reference 16); patient A_3 ●···● and patient B_5 □—□ with low monocyte insulin binding (see reference 11). Shaded area is ±2 SEM for data from 13 normal individuals.

show significant correlations. This may reflect the uncertainty involved in deriving such parameters from a limited number of data points.[17] In our experience, there is more variability in insulin binding to erythrocytes than monocytes at the limiting, low bound/free ratios of ^{125}I-insulin. This variability in turn affects the affinity profiles derived from these data. (4) In situations where insulin binding deviates greatly from normal, such as in patients with the syndromes of extreme insulin resistance and acanthosis nigricans, there is a good correlation between percent specific ^{125}I-insulin bound to the two cell types. Again, derived parameters such as R_0 and $\bar{K}_e$ in these patients are difficult to reliably determine, particularly in erythrocytes. (5) Insulin binding to erythrocytes may change more slowly with a change in metabolic status, as was found in the insulin-dependent diabetics who went from poor to improved diabetic control. This may result from differences in the way these cells are regulated, or by regulation of only a limited subpopulation of circulating erythrocytes as previously suggested.[6] If this were true, the erythrocyte may well reflect the same metabolic changes as the monocyte, but differences in insulin binding to erythrocytes would appear over a more extended time. Similar conclusions were reached by Spanheimer et al. in their study of erythrocyte and monocyte binding in obese patients undergoing starvation.[18] (6) While variability in insulin binding at the limiting low bound/free ratio of ^{125}I-insulin may obscure correlations of R_0 and $\bar{K}_e$ as discussed above, we find that the variance for tracer binding is similar between erythrocytes and monocytes (Figure 1). De Pirro[19] also found a similar degree of variability in tracer binding to the two cell types in normal subjects. In contrast, obese patients were found to have a much greater variability in tracer binding to erythrocytes than monocytes. Thus, discrimination between the two groups of subjects was less clear when using erythrocytes rather than monocytes.[19]

Technically, there is no simple way of isolating a subpopulation of erythrocytes with a well-defined mean cell age. There are no data available on the degree of prior phlebotomy that might significantly reduce erythrocyte mean cell age and hence increase insulin binding. Neither the reticulocyte content[5] nor the pyruvate kinase activity[6] of a single blood sample can be converted into a specific mean cell age of the sample. The predicted half-life of an erythrocyte preparation, based on red cell pyruvate kinase activity, has been shown statistically to be no different from that observed using Cr^{51} labeling of red cells.[20] However, technical problems limit the usefulness of radiochromium if it is used to estimate erythrocyte mean cell age. In our opinion, since a simple and reliable method of estimating the erythrocyte mean cell age of an individual subject is not available, there is no meaningful way to correct erythrocyte insulin binding for variability in mean cell age of the preparation assayed. Certainly, some caution is properly advised when interpreting data on insulin binding to red cells of patients with a slightly subnormal erythrocyte survival.[21–23] Monocyte studies are appropriate to corroborate conclusions drawn from erythrocyte insulin binding in patients known to have shortened red cell survival or increased red cell turnover.

Our results on the effects of corticosteroid administration on erythrocyte insulin binding differ from those reported by Yasuda and Kitabchi.[24] Before ascribing these differences to experimental design, sex of subjects, or dose and type of steroid, it should be pointed out that three extant monocyte studies of subjects treated in a similar fashion with steroids arrived at different conclusions.[10,25,26] Thus, studies of the effects of steroids on insulin binding to blood cells remain incomplete and confusing.

Some recent reports allow comparison between monocyte and erythrocyte insulin binding data obtained from the same individuals.* An effect of the menstrual cycle on insulin binding to monocytes, but not red cells, has been found.[27] No correlations between red cell and monocyte insulin binding in pregnant women near term or in women on birth control pills were found.[28] Changes in the affinity of the insulin receptor were the same in monocytes and erythrocytes in response to glucose and insulin infusions,[29] as well as exercise.[30] A general correlation between red cell and monocyte insulin binding was found in studies of a group of nine patients with lipoatrophic diabetes.[31]

We would prefer to measure insulin receptors in all major target tissues of man, but this is not practical at present. Blood cells, therefore, continue to offer a feasible alternative. Preliminary studies suggest that receptor-mediated endocytosis, which may be a major mechanism of receptor regulation,[32] is similar in hepatocytes and monocytes. At this time, we suggest that insulin binding studies in monocyte may be more discriminating than those in erythrocytes, particularly after an acute metabolic perturbation. Certainly, simultaneous erythrocyte and monocyte insulin binding studies yield the greatest amount of useful information about the insulin receptor status of patients.

ACKNOWLEDGMENT

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* Only comparative studies involving simultaneous studies of erythrocyte and monocyte binding are discussed in this report.

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