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Expression of functional insulin-like growth factor-1 receptor on lymphoid cell subsets of rats

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SUMMARY

It has become evident that insulin-like growth factor-1 (IGF-1) acts as a growth factor for immune cells, yet the precise regulatory role of IGF-1 in the immune system is unknown. The aim of this study was to examine the distribution of IGF-1 receptors on rat lymphoid cells. A flow cytometric method was used, with a biotinylated and functionally active IGF-1 analogue, namely des(1-3)IGF-1, which binds well to IGF-1 receptor but poorly to IGF binding proteins, followed by phycoerythrin-conjugated streptavidin (PE–SA) staining. Our results showed that IGF-1 receptors were readily detectable on a wide variety of the immune cells, including T cells, B cells and monocytes, but the binding capacity for IGF-1 was monocytes > B cells > T cells, as determined by titration experiments. Furthermore, the level of expression on resting $CD4^+$ T lymphocytes was greater than on $CD8^+$ cells, and the concentration of biotin–des(1-3)IGF-1 required to demonstrate the binding to IGF-1 receptor on $CD8^+$ cells (68 nmol/l) was 200-fold higher than for $CD4^+$ cells (0·34 nmol/l), indicating that most of the IGF-1 receptor on $CD8^+$ cells represented lower affinity sites. The level of IGF-1 receptor expression was increased several-fold after concanavalin A stimulation on both $CD4^+$ and $CD8^+$ T-cell subsets. Kinetic analysis of the expression of IGF-1 receptor and its association with interleukin-2 receptors (IL-2R) following activation showed a similar pattern, with no significant differences in the ratio of IGF-1 receptor : IL-2R per cell during the 3 days of cell culture. Our studies suggest that biological activities of IGF-1 include direct stimulation of immune cells, and that expression of IGF-1 receptor may have a role in regulation of T-cell function.

INTRODUCTION

Insulin-like growth factor-1 (IGF-1) is a 7500 MW polypeptide that has well-characterized growth-promoting activities in a wide variety of cell types in culture. IGF-1 has been shown to act on the immune system *in vivo* and to enhance the mitogenic response of lymphocytes *in vitro*.[1–4]

Receptors for IGF-1 have been found in numerous tissues, including lymphoid tissues.[5] Two types of IGF receptor have been identified.[6] The type 1 IGF receptor, which consists of two peptide-binding α subunits and two membrane-spanning β subunits, has intrinsic tyrosine kinase activity and mediates growth responses to both IGF-1 and IGF-2. The type 2 IGF receptor is a single-chain polypeptide that binds only to IGF-2; its function is currently unknown. At high concentration IGF-1 can interact with the insulin receptor.[7] In addition, IGF-1 also binds to multiple IGF binding proteins (IGFBP) present in serum. Recently, cell membrane-associated IGFBP have been documented and the potential IGF-1 binding sites on the cell surface may, therefore, represent in part IGFBP rather than specific IGF-1 receptors.[8]

In order to characterize the mechanism of action of IGF-1 on the immune system, we set out to analyse the expression of functional IGF-1 receptors on lymphoid subpopulations, using a biologically active and biotinylated IGF-1 analogue, namely des(1-3)IGF-1, which binds to IGF-1 receptors with affinity equal to that of IGF-1 but binds poorly to IGFBP. A multi-parameter flow cytometric approach was used, providing a specific and sensitive approach for the detection of IGF-1 receptors, together with the ability to analyse the distribution and relative affinity of IGF-1 receptors expressed on different cell types in heterogeneous cell populations.

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Abbreviations: CD, cluster of differentiation; Con A, concanavalin A; DMF, dimethylformamide; FACS, fluorescence-activated cell sorter; FITC, fluorescein isothiocyanate; IGFBP, IGF binding proteins; IGF-1, insulin-like growth factor-1; PE–SA, phycoerythrin–streptavidin.

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MATERIALS AND METHODS

Biotin-labelling of des(1-3)IGF-1

Des(1-3)IGF-1 was purchased from GroPep (Adelaide, Australia) and biotinylated using the BIOTIN-X-NHS kit

(catalogue no. 203187; Calibiochem, San Francisco, CA). Briefly, prior to biotin-labelling, 1 mg of des(1-3)IGF-1 was dissolved in 100 μl of 10 nM HCl and diluted to a concentration of 1 mg/ml using 0·1 M $NaHCO_3$. Freshly dissolved 250 μl 0·1 M biotin-*N*-hydroxy-succinimydyl ester (biotin-NHS) analogue, biotin-X-NHS, in dimethylformamide (DMF) was added to 1 ml of des(1-3)IGF-1 solution to yield an approximately 150-fold molar radio of biotin to des(1-3)IGF-1. After 60 min incubation at room temperature with gentle agitation, biotin–des(1-3)IGF-1 was separated from free reagent by dialysing several times overnight at 4° against phosphate-buffered saline (PBS) (pH 7·3) in a dialysing tube (Spectra/Pro; MWCO:3500, Spectrum Medical Industries Inc., Los Angeles, CA), aliquoted, lyophlized, and stored at −70° before use. The biotinylation efficiency of this method was about 90%, as determined by absorption to agarose beads conjugated with streptavidin (Sigma) and measuring the des(1-3)IGF-1 concentration left in the supernatant by radioimmunoassay, as described previously.[9] Biological activity of biotin–des(1-3)IGF-1 was measured by protein synthesis of L6 myoblasts after biotin–des(1-3)IGF-1 stimulation, as described previously.[10] Briefly, L6 myoblasts were cultured in serum-free DMEM medium containing 0·9 μCi [^{3}H]leucine in the presence of biotin–des(1-3)IGF-1 or des(1-3)IGF-1 at serial concentrations. After 18 hr incubation at 37° in a 5% CO_2 incubator, cells were washed with PBS, treated with 5% trichloracetic acid (TCA), and solubolized by 0·5 M NaOH containing 0·1% Triton-X-100. The [^{3}H]leucine incorporation of TCA-precipitated cellular protein was then measured by a β-scintillation counter (LKB Pharmacia, Uppsala, Sweden).

Preparation of rat lymphocytes

Male and female hooded Wistar rats (weight 150–200 g) were obtained from the animal house of the CSIRO Division of Human Nutrition (Adelaide, Australia). Rat splenocytes were obtained by cutting fresh spleen into small fragments and gently passing through a stainless steel mesh. The cells were further purified by density gradient centrifugation using Lymphoprep™ (density 1·077; Nycomed Pharma AS, Oslo Norway) at 600 *g* for 20 min at room temperature. Cells from the interface were collected, washed twice in PBS and resuspended in RPMI-1640 medium. Cell recovery and viability was determined by trypan blue exclusion using a haemocytometer. For analysis of expression of IGF-1 and interleukin-2 receptors (IL-2R) on activated T cells, resting splenocytes (1×10^6/ml) were cultured in RPMI-1640 medium containing 10% fetal calf serum in the presence of concanavalin A (Con A; 5 μg/ml) at 37° in a 5% CO_2 incubator, and activated T cells were then collected at the times indicated.

Analysis of IGF-1 receptor distribution

To detect IGF-1 receptor expression, unfractionated splenocytes were initially incubated in serum-free RPMI-1640 for 1 hr at 37° to remove or endocytose membrane-bound IGF-1. Cells (1×10^6) in 200 μl of ligand binding buffer (100 nM HEPES, 30 mM NaCl, 5 mM KCl, 1·2 mM $MgSO_4$, 8·8 mM dextrose, 1% bovine serum albumin, 0·1% NaN_3, pH 7·6) were then incubated with serial concentrations of biotin–des(1-3)IGF-1 for 2 hr on ice. NaN_3 was included to optimize ligand binding without significant internalization of the ligand–receptor complex.[11] Non-specific binding of biotin–des(1-3)IGF-1 was determined by competitive inhibition with a 100-fold excess of unconjugated IGF-1. After incubation, cells were washed once with PBS–azide and incubated with monoclonal antibodies (mAb) against rat cell-surface antigens (Table 1) for 30 min on ice. After two washes, the cells were incubated on ice with a pretitrated amount of phycoerythrin (PE)-conjugated streptavidin (PE–SA; Dako, Glostrup, Denmark) and $F(ab')_2$ fragments of fluorescein isothiocyanate (FITC)-conjugated sheep anti-mouse immunoglobulin (Silenus, Melbourne, Australia) for 30 min in the dark. The cells were again washed and resuspended in 200 μl of fixative buffer (PBS containing 2% D-glucose and 2·6% formaldehyde).

Table 1. Description of mAb to rat cells used in this study

Surface markers	mAb	Reactivity	Source
T-cell receptor	R73	$\alpha\beta$ T-cell receptor	Serotec
CD4	W3/25	T-helper cells	Serotec
CD8	OX8	Cytotoxic T cells, natural killer cells	Serotec
CD45 (B-cell form)	OX33	Leucocyte common antigen present only on B cells	Serotec
Monocyte/macrophage	ED-1	Monocytes, macrophages, Dendritic cells	Serotec
CD25	OX39	IL-2R α-chain	Serotec
Negative control	X63	Unknown specificity	FMC

Serotec (Oxford, UK).
FMC, Flinders Medical Centre, Adelaide, South Australia.

Flow cytometric analysis

Stained cells were analysed by flow cytometry (FACScan; Becton Dickinson, Mountain View, CA). Briefly, 10 000 cells were counted, and the mean fluorescence intensity of PE staining was then determined by setting the gate on the respective FITC-positive populations. The fluorescence intensity of cells incubated with a 100-fold excess of unlabelled IGF-1, as a control for non-specific binding of biotin–des(1-3)IGF-1, was < 10% of biotin–des(1-3)IGF-1 staining alone. Cells staining with PE–SA alone and isotype-specific mAb of irrelevant specificity were used to assess background staining.

RESULTS

Specificity and biological activity of biotin-des(1-3)IGF-1

It has been reported that approximately 10% of the total IGF-1 binding is due to IGFBP on the cell surface.[8] The N-terminally truncated IGF-1 analogue, des(1-3)IGF-1, which binds poorly to IGFBP but binds to IGF-1 receptors with equal affinity to IGF-1,[12] was therefore used to determine the specific binding for IGF-1 receptor on the cell surface. In addition, since IGF-1 also binds to insulin receptor at high concentrations, the specificity of des(1-3)IGF-1 binding to IGF-1 receptor was confirmed in IM-9 cells, a cell line known to express both insulin and IGF-1 receptors (insulin: IGF-1 receptor ratio, 3·5:4·5).[13] As shown in Fig. 1, a 100-fold excess of unlabelled insulin did not significantly inhibit the binding of biotin-labelled des(1-3)IGF-1 to IM-9 cells, whereas unlabelled des(1-3)IGF-1 almost completely diminished tracer binding,

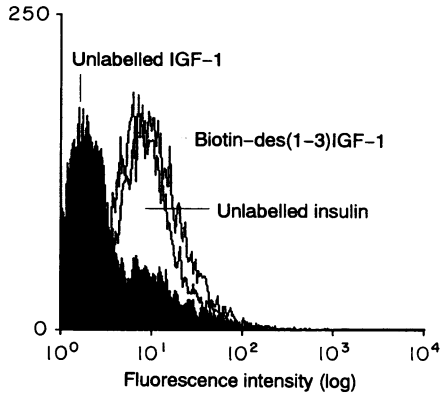

Figure 1. Binding specificity of biotin–des(1-3)IGF-1. IM-9 cells, a cell line known to express both insulin and IGF-1 receptors, were stained with 1 ng of biotin–des(1-3)IGF-1 alone or in the presence of 100-fold excess of unlabelled des(1-3)-IGF-1 or unlablled insulin as indicated. The cells were subsequently stained with PE–SA. A histogram profile of PE fluorescence is displayed.

indicating specificity of biotin–des(1-3)IGF-1 for the IGF-1 receptors. Because biological activities of growth factors depend on their binding to the functional receptors, we next determined the functional activity of biotin–des(1-3)IGF-1 by measuring the protein synthesis of rat L6 myoblasts after biotin–des(1-3)IGF-1 stimulation. As shown in Fig. 2, the recovery of biological activity of biotin–des(1-3)IGF-1 was up to 80% compared with des(1-3) IGF-1, indicating that biotin–des(1-3)IGF-1 binds mainly to its functional receptors.

Detection of IGF-1 receptor expression on unstimulated splenocytes

To examine IGF-1 receptor distribution on specific lymphoid cell types, unfractionated splenocytes were dual-labelled with PE–SA–biotin–des(1-3)IGF-1 and FITC-indirectly labelled mAb against T cells, B cells and monocytes. The intensity of

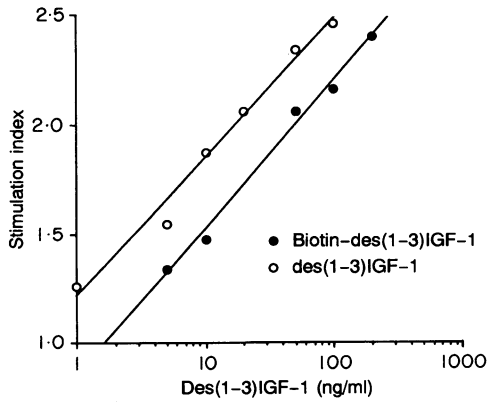

Figure 2. Biological activity of biotin–des(1-3)IGF-1. Rat L6 myoblasts were cultured in a serum-free medium containing 0·9 μCi [^{3}H]leucine in the presence of des(1-3)IGF-1 or biotin–des(1-3)IGF-1 at the concentration indicated. After 18 hr incubation at 37° in a CO_2 incubator, the [^{3}H]leucine incorporation was determined as described in the Materials and Methods. Results are expressed as a stimulation index (the ratio of growth factor/medium) for protein synthesis.

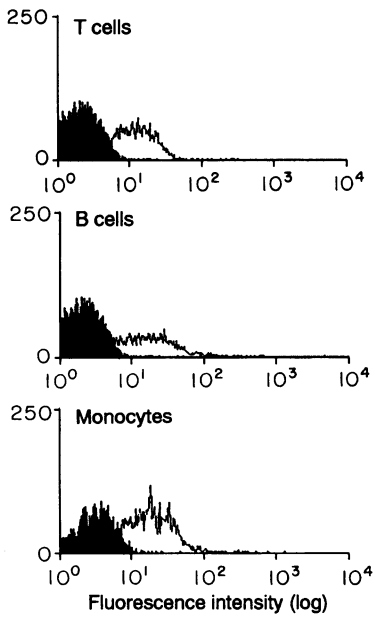

Figure 3. Expression of IGF-1 receptors on rat lymphoid subsets. Rat splenocytes (1×10^6) were stained with 68 nmol/l of biotin–des(1-3)IGF-1 and PE–SA, as well as counterstained with FITC-indirectly conjugated mAb against T cells, B cells and monocytes. Stained cells were analysed by FACScan as described in the Materials and Methods. The open histograms represent biotin–des(1-3)IGF-1 and PE–SA staining, whereas the closed histograms are cells stained with PE–SA only.

PE fluorescence representing IGF-1 receptor in different subsets was determined by setting an appropriate gate on the total of the respective FITC-positive cells. As illustrated by the flow cytometric histograms from one experiment (representative of four experiments) in Fig. 3, the IGF-1 receptor was detectable on unstimulated T and B lymphocytes as well as

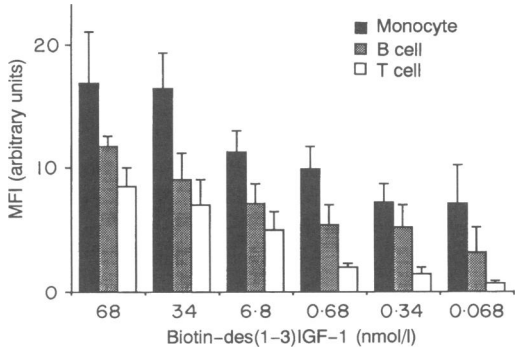

Figure 4. Detection of IGF-1 receptors on rat T cells, B cells or monocytes. Rat splenocytes (1×10^6) were incubated with biotin–des(1-3)IGF-1 at the indicated concentrations. After staining with PE–SA, the cells were counterstained with FITC-indirectly conjugated mAb against T cells, B cells and monocytes and analysed on FACScan as described in the Materials and Methods. Mean fluorescence intensities (MFI) were measured for IGF-1 receptor expression in the respective FITC-positive cells. Results are expressed as the specific MFI, which was calculated by subtracting the MFI of cells that had been incubated with biotin–des(1-3)IGF-1 in the presence of a 100-fold excess of unlabelled des(1-3)IGF-1 as a control for non-specific fluorescence.

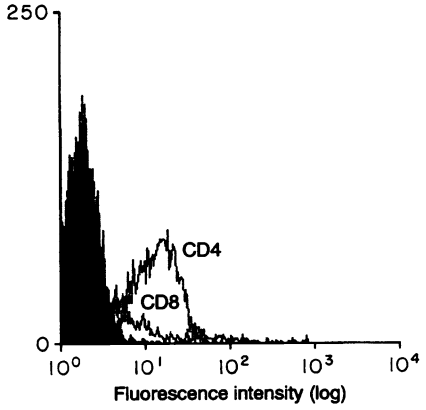

Figure 5. Expression of IGF-1 receptors on rat $CD4^+$ and $CD8^+$ T cells. Rat splenocytes were dual-labelled with biotin–des(1-3)IGF-1 followed by PE–SA and FITC-indirectly conjugated mAb to $CD4^+$ and $CD8^+$ T cells, as indicated in Fig. 3. The open histograms represent biotin–des(1-3)IGF-1 and PE–SA staining, and the closed histograms are cells stained with PE–SA only.

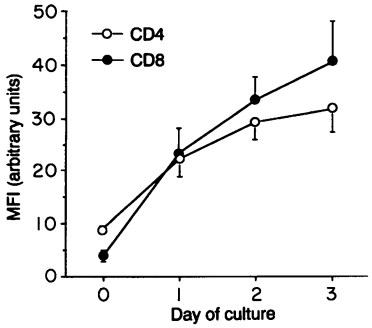

Figure 7. Up-regulation of IGF-1 receptor expression on T-cell subsets by Con A stimulation. Rat splenocytes (1×10^6/ml) were cultured in the presence of 5 μg/ml of Con A for the times indicated. After washing with serum-free medium, the cells were stained for IGF-1 receptor expression in $CD4^+$ or $CD8^+$ T cells, and results are expressed as the specific mean fluorescence intensities by subtracting the non-specific binding as indicated in Fig. 4. The percentage of $CD4^+$ and $CD8^+$ cells during the cell culture period is shown in Table 2.

monocytes. Furthermore, to analyse whether the IGF-1 binding sites on these cells represented relatively high- or low-affinity IGF-1 receptor or both, unfractionated splenocytes were incubated with 0·068–68 nmol/l of biotin–des(1-3)IGF-1 and stained with PE–SA. Cells were then labelled with mAb against respective surface markers. The mean PE fluorescence intensity of IGF-1 receptors within FITC-positive cells was then measured for each biotin–des(1-3)IGF-1 concentration. Non-specific binding was determined by competitive inhibition with 100-fold excess of unlabelled IGF-1, and subtracted to yield specific fluorescence staining. As shown in Fig. 4, IGF-1 receptor-positive monocytes were detectable at the lowest biotin–des(1-3)IGF-1 concentration tested, 0·068 nmol/l, whereas maximal specific binding was achieved at a concentration of aproximately 34 nmol/l. On the other hand, IGF-1 receptors were barely detectable on T cells after staining with up to 0·34 nmol/l biotin–des(1-3)IGF-1, and saturation of binding did not appear to be achieved at the highest biotin–des(1-3)IGF-1 concentration tested, 68 nmol/l. B cells showed a binding character intermediate between that of monocytes and T cells. The relatively high biotin–des(1-3)IGF-1 concentration required to saturate IGF-1 receptors on T or B cells compared with monocytes suggests that most IGF-1 receptors on these cells bind to IGF-1 with low affinity, whereas monocytes appear to display predominantly higher-affinity IGF-1 receptors.

Table 2. The percentage of $CD4^+$ and $CD8^+$ cells in Con A-stimulated splenocyte culture

	Time–course of Con A stimulation			
T-cell subset	Day 0	Day 1	Day 2	Day 3
$CD4^+$ (%)	39·2 ± 3·5	37·5 ± 4·2	38·3 ± 2·4	40·2 ± 1·4
$CD8^+$ (%)	23·3 ± 6·0	24·5 ± 8·0	21·2 ± 6·5	20·3 ± 7·5

We further investigated the expression of IGF-1 receptors on resting T-cell subsets, namely $CD4^+$ and $CD8^+$ T cells, by using the same staining technique. The results showed that the level of IGF-1 receptors on $CD4^+$ T cells was much greater than $CD8^+$ cells (Fig. 5). In addition, the requirement of biotin–des(1-3)IGF-1 for a detectable level of IGF-1 receptors on $CD8^+$ cells (68 nmol/l) was 200-fold higher than $CD4^+$ cells (0·34 nmol/l), indicating that $CD8^+$ cells express IGF-1 receptor with a lower affinity than $CD4^+$ cells (Fig. 6).

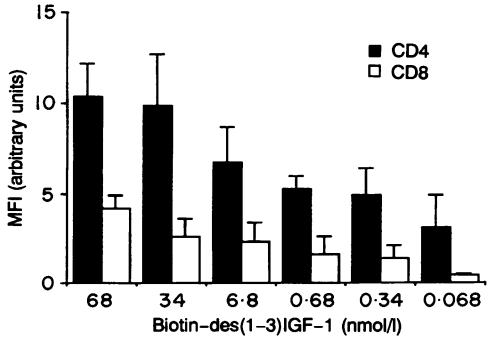

Figure 6. Detection of IGF-1 receptors on rat $CD4^+$ and $CD8^+$ T cells. Rat splenocytes were stained for IGF-1 receptor expression in $CD4^+$ or $CD8^+$ T cells, as described in Fig. 4.

Up-regulation of IGF-1 receptor expression on T-cell subsets by Con A stimulation

Previous studies in the human involving radiolabelled ligand binding assays have shown that activation of T cells by mitogens *in vitro* increases the number of IGF-1 binding sites.[4,14] However, it is not clear whether the up-regulation of IGF-1 receptor expression is restricted to a particular T-cell subset when unfractionated T cells are activated. We therefore examined the expression of IGF-1 receptor on Con A-activated $CD4^+$ or $CD8^+$ T cells *in vitro* by the dual-labelling technique. As shown in Fig. 7, IGF-1 receptor expression on both $CD4^+$ and $CD8^+$ cells was up-regulated following Con A stimulation; the fluorescence intensity of IGF-1 binding to $CD4^+$ cells was increased threefold on day 2 and appeared to reach a plateau on

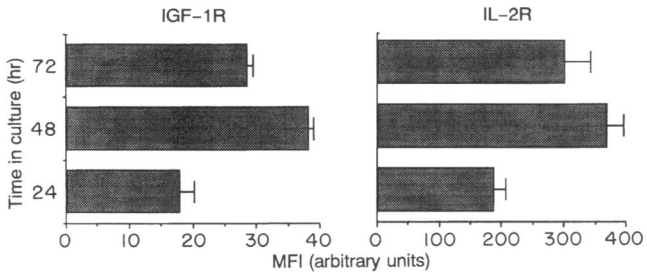

Figure 8. Co-expression of IGF-1 receptor and IL-2R on activated T cells. Rat splenocytes were stimulated by Con A under the conditions described in Fig. 7. Cells were collected at the indicated times and dual-labelled with PE–SA–biotin–des(1-3)IGF-1 for IGF-1 receptor expression and FITC-conjugated mAb against IL-2R, as described in the Materials and Methods. The specific mean fluorescence intensities of IGF-1 receptor expression in IL-2R$^+$ cells are shown.

day 3. The level of IGF-1 receptor on CD8$^+$ cells was increased 10-fold by day 3 and did not plateau over the 3-day culture period. The different pattern of IGF-1 receptors on CD4$^+$ and CD8$^+$ cells after activation was not due to changes of ratio of CD4$^+$ to CD8$^+$ cells during cell activation (Table 2).

It is well-known that T-cell activation and proliferation is dependent on a critical threshold level of expression of IL-2R. We next determined the co-expression of IGF-1 receptor and IL-2R (CD25) on Con A-stimulated T cells. Expression of both receptors showed virtually identical patterns during the 3-day incubation (Fig. 8). In both cases, receptor expression was increased on day 1 and doubled on day 2, and then reduced slightly on day 3 following Con A stimulation, so that the ratio of IL-2R : IGF-1 receptor did not significantly change with the time–course of Con A stimulation (day 1, $12{\cdot}1 \pm 1{\cdot}2$; day 2, $10{\cdot}6 \pm 0{\cdot}8$; day 3, $10{\cdot}6 \pm 1{\cdot}2$).

DISCUSSION

Accumulated evidence from recent studies has shown that IGF-1 stimulates growth of lymphoid tissues *in vivo*,[1,2] and enhances mitogenic responses of lymphocytes *in vitro*.[3,4] However, the precise role of IGF-1 in the regulation of immune responses is still unclear. Using a radiolabelled IGF-1 binding assay, several studies have demonstrated binding sites for IGF-1 on human lymphoid cells such as T or B lymphomas, as well as resting or activated peripheral lymphocytes.[5] Because IGF-1 also binds to IGFBP, which are produced by lymphoid cells[15] and may also be bound to the cell surface,[8] the IGF-1 binding sites determined by IGF-1 binding assay could represent a surface protein other than the IGF-1 receptor. In addition, IGF-1 ligand binding assays do not provide insight into the distribution of receptors in highly heterogeneous cell populations such as the immune system.

In this study, we have used a biologically active biotin-labelled IGF-1 analogue, des-(1-3)IGF-1, combined with multiparameter flow cytometry to examine the distribution of the IGF-1 receptor on phenotypically defined subsets of rat spleen cells. A major advantage of this technique is that des-(1-3)IGF-1 binds poorly to IGF binding proteins but normally to IGF-1 receptors,[12] thereby providing a more specific assay. Moreover, detection of receptor expression via binding of the biologically active and labelled ligand itself provides a more reliable estimate for the functional IGF-1 receptor than the use of antibodies for individual receptor subunits.

Our results, in agreement with previous studies in the human,[14,16] showed that IGF-1 receptors were detectable on unstimulated monocytes, B cells, T cells and subsets. However, the results of titration experiments showed that the relative binding affinity of IGF-1 receptor was monocytes > B cells > T cells. Furthermore, comparative analysis of IGF-1 receptor expression on T-cell subsets led to the observation that CD4$^+$ cells had higher IGF-1 receptor expression than CD8$^+$ cells. The concentrations of biotin–des(1-3)IGF-1 required for a detectable level of IGF-1 receptors on CD8$^+$ cells (68 nmol/l) were 200-fold higher than CD4$^+$ cells (0·34 nmol/l), suggesting that CD8$^+$ cells express IGF-1 receptors with a lower affinity. Thus, the finding of significant expression of IGF-1 receptor on monocytes and CD4$^+$ cells would be consistent with a direct biological effect of IGF-1 on these cells, as suggested by the observation that IGF-1 enhances myeloid cell proliferation *in vitro*[17] and increases CD4$^+$ cell numbers *in vivo* (D. Belford, J. G. Robertson, X. Xu, F. M. Tomas & K. T. Pickering, unpublished data).

Although up-regulation of IGF-1 binding sites on activated T cells and enhancement of T-cell proliferation by IGF-1 have been observed by a number of groups,[4,14,18] the precise mechanism by which IGF-1 stimulates T-cell proliferation is still unclear. Therefore, we were interested in IGF-1 receptor expression within T-cell subsets and its correlation with IL-2R expression after mitogen stimulation. The results showed that while Con A-stimulated up-regulation of IGF-1 receptor was observed in both CD4$^+$ and CD8$^+$ T cells, the fluorescence intensity of IGF-1 binding to CD4$^+$ cells was increased threefold on day 2 and reached a plateau on day 3, whereas the level of IGF-1 receptor on CD8$^+$ cells was increased 10-fold by day 3 and had not reached a plateau. Moreover, the different patterns of IGF-1 receptor expression were not due to the changes of ratio of CD4$^+$ to CD8$^+$ cells during cell activation.

We also looked at correlations between IGF-1 receptor and IL-2R expression following Con A stimulation because the expression of IL-2R is a crucial event in T-cell activation and proliferation.[19,20] Con A-activated rat T cells were used to compare the time–courses of induction of IGF-1 receptors and IL-2R. Our data showed that the pattern of IGF-1 receptor expression was similar to that of IL-2R and the ratio of IL-2R : IGF-1 receptor per cell was not significantly changed during the time–course of Con A stimulation. The close temporal relationship between the expression of IL-2R and IGF-1 receptor suggests that expression of the two receptors may be important in signal transduction for T-cell proliferation. One possibility is that growth signals induced by IGF-1 receptor may complement the IL-2R-induced signals for T-cell growth, in which IGF-1 receptors may function like the receptors for 'classical' growth factors, such as epidermal growth factor (EGF) receptor involved in signal transduction for DNA synthesis, but not for cell cycle transiting, which is dependant on IL-2R-derived signals.[21] Thus, co-expression of IGF-1 receptor may have potential implications for proliferative signal transduction for T cells through a mechanism by which IGF-1 receptor and IL-2R may utilize overlapping but distinct signalling pathways for T-cell proliferation.

Taken together, our results support the hypothesis that

IGF-1 has a potential regulatory role in the immune system, and further indicate that the effect of IGF-1 could be directly at the level of monocytes and B and T cells.

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