Differential signalling for enhanced hexose uptake by interleukin (IL)-3 and IL-5 in male germ cells

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We studied the expression and function of the IL (interleukin)-3 and IL-5 family of receptors in male germ cells. RT (reverse transcription)-PCR showed expression of mRNAs encoding the α and β subunits of the IL-3 and IL-5 receptors in human testis, and the presence of IL-3 and IL-5 receptors α and β proteins was confirmed by immunoblotting with anti- α and anti- β antibodies. The immunolocalization studies showed expression of these receptors in the germ line in the human testis and in human and bovine ejaculated spermatozoa. Functional studies with bull spermatozoa indicated that IL-3 signalled for increased uptake of hexoses in these cells at picomolar concentrations compatible with expression of functional high-affinity IL-3 receptors in these cells. In

contrast, IL-5 failed to induce increased hexose uptake in bull spermatozoa. Experiments using HL-60 eosinophils that express functional IL-3 and IL-5 receptors confirmed that IL-3, but not IL-5, signalled for increased hexose uptake. Our findings suggest that differential signalling for increased hexose uptake by heteromeric high-affinity IL-3 and IL-5 receptors in mammalian spermatozoa is a property that depends on the identity of the α -subunit forming part of the $\alpha\beta$ -complex and is not a property specific to the germ cells.

Key words: glucose transport, HL-60 cells, interleukin-3 receptor, interleukin-5 receptor, spermatozoa.

INTRODUCTION

IL (interleukin)-3, IL-5 and GM-CSF (granulocyte/macrophage colony-stimulating factor) belong to a family of cytokines that regulate the proliferation, survival and differentiation of haematopoietic cells via interaction with specific cell-surface receptors. There are two types of receptors for each cytokine that show high- or low-affinity for the ligand, depending on their subunit composition. The high-affinity receptors are $\alpha\beta$ -heterocomplexes composed of α - and β -subunits that bind their cognate ligands with high affinity, with a K_D in the picomolar range [1]. In the $\alpha\beta$ -heterocomplex, the ligand specificity is dictated by the identity of the α -subunit, while the β -subunit is common to the three receptors. The isolated α -subunit behaves as a low-affinity receptor, which binds its ligand with a K_D in the low nanomolar range. There are three different α -subunits that show no significant similarity in primary structure and show absolute specificity for their respective ligands.

GM-CSF receptors have been identified on most types of myeloid progenitors and on mature monocytes, neutrophils, eosinophils, basophils, dendritic cells and tumour cell lines [2–4]. IL-3 receptors are present on early haematopoietic progenitor cells, on certain committed myeloid progenitors, eosinophils and basophils and in brain [5]. In contrast with this wide distribution, IL-5 receptor expression appears to be restricted to cells such as eosinophils [2].

GM-CSF, IL-3 and IL-5 exert their effects on the proliferation, survival and differentiation of haematopoietic cells by signalling through high-affinity $\alpha\beta$ -receptor complexes, and show a number of overlapping biological activities. It is commonly accepted that the β -subunit plays a central role in signalling through the high-

affinity receptor; however, the α -subunit has a functional role that goes beyond that of a component that provides the necessary structural scaffold for ligand binding. In most cells, both high- and low-affinity receptors co-exist, which indicates greater expression of α - compared with β -subunits, but there are also examples of cells that express isolated α -subunits without evidence of high-affinity receptors. A direct functional role for the α -subunit of the GM-CSF receptor has been shown in studies showing enhanced glucose uptake in response to GM-CSF in melanoma cells that express a low-affinity GM-CSF receptor [6], and in *Xenopus laevis* oocytes that express an isolated α -subunit [7]. It has also been shown that in mice, pre-implantation embryos express an isolated α -subunit that is capable of signalling for increased glucose uptake and enhanced proliferation [8].

GM-CSF signalling for increased uptake of glucose and vitamin C has been demonstrated in human host defence cells [9] and in mouse bone marrow cells [10]. Similarly, IL-3 increases glucose transport in myeloid cells [11], confirming that GM-CSF and IL-3 exhibit overlapping biological activities in haematopoietic cells due to a similar pattern of receptor expression. We have shown that non-haematopoietic cells, such as bovine spermatozoa, express functionally active low- and high-affinity GM-CSF receptors that signal for increased transport of glucose and vitamin C [12].

In the present study, we evaluated the expression of IL-3 and IL-5 receptors in human and bovine male germ cells and determined the effect of these cytokines on glucose transport in bovine spermatozoa. Our data indicated that human and bovine germ cells express receptors for IL-3 and IL-5, and that IL-3, but not IL-5, signalled for increased glucose uptake in bovine spermatozoa and in human myeloid cells.

Abbreviations used: DOG, 2-deoxy-D-glucose; GM-CSF, granulocyte/macrophage colony-stimulating factor; IL, interleukin; RT, reverse transcription; TNF α , tumour necrosis factor α .

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MATERIALS AND METHODS

Sample collection

Human semen was collected in sterile plastic containers from healthy young men [13]. The ethical approval was obtained by consent form according to the regulations of the Ethics Committee from the Universidad Austral de Chile. Bovine spermatozoa ejaculates were obtained from the Centro de Inseminación Artificial, Universidad Austral de Chile, as indicated previously [12,15,18].

Cell culture

HL-60 eosinophils were cultured in IMDM (Iscove's modified Dulbecco's medium):10 % (v/v) foetal calf serum containing glutamine and streptomycin/penicillin [9,14]. Viability was measured by Trypan Blue exclusion and was always greater than 95 %.

RT (reverse transcription)-PCR

Total human testis RNA was obtained from Clontech Laboratories (Palo Alto, CA, U.S.A.). Single-stranded cDNA synthesis and PCR were carried out as previously described [12]. Primers were as follows: IL-3 receptor α -subunit primer: 5'-ACAGGTCAGAGACAGAACCTCC-3', position 902–923, and 5'-CTGTTCTTCTTCCTGGCAGC-3', position 1457–1438; IL-5 receptor α -subunit primer: 5'-GCCAAGAATACAGCAAAGACA-3', position 794–814, and 5'-CCCACATAAATAGGTTGGCTC-3', position 1224–1244; Common β -subunit primer: 5'-CTACAAGCCAGCCAGATGC-3', position 859–879, and 3'-ACCCGTAGATGCCACAGAAGC-5', position 1390–1410. The PCR conditions were 94 °C for 1 min and 65 °C for 2 min for 35 cycles. PCR products were separated by 1.5 % (w/v) agarose gel electrophoresis and visualized by staining with ethidium bromide.

Uptake assays

Uptake assays in spermatozoa and HL-60 cells were measured as described previously [9,15]. The cells were suspended in incubation buffer (15 mM Hepes, pH 7.6, 135 mM NaCl, 5 mM KCl, 1.8 mM CaCl₂ and 0.8 mM MgCl₂), washed by centrifugation at 1500 *g* for 5 min in the same buffer and resuspended at 2×10^7 cells/ml for HL-60 cells and at 5×10^8 cells/ml for spermatozoa. 2-Deoxy-D-glucose (DOG) uptake assays were performed in a final volume of 0.25 ml of incubation buffer containing 2×10^6 cells for HL-60 cells and 1×10^8 cells for spermatozoa, 1 μ Ci of 2-[1,2-³H(N)]-deoxy-D-glucose (specific activity, 26.2 Ci/mmol; NEN-Dupont, MA, U.S.A.) and 0.3–20 mM DOG. The cells were washed twice with cold PBS, lysed in 10 mM Tris/HCl (pH 8.0) containing 0.2 % (w/v) SDS, and the incorporated radioactivity was determined by liquid-scintillation counting. When appropriate, the cells were pre-incubated with recombinant human IL-3, IL-5, GM-CSF or TNF α (tumour necrosis factor α) as indicated in the Figure legends.

Immunocytochemistry

For immunoperoxidase localization, spermatozoa and human testis sections from archived paraffin-embedded tissue blocks from the Hospital Regional de Valdivia were treated as previously described [12] using specific antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A.). As controls, cells and sections were incubated with antibodies reabsorbed with the respective peptide used to generate the antibodies. Cells and sections were counterstained with haematoxylin.

Immunoblotting

Spermatozoa membrane proteins were obtained as previously described [15] and resolved by SDS/PAGE (30 μ g/lane) in a 10 % (w/v) polyacrylamide gel and transferred on to Immobilon (Millipore, Bedford, MA, U.S.A.). The antibody blots were developed by ECL[®] (enhanced chemiluminescence; Amersham Biosciences, Arlington Heights, IL, U.S.A.).

RESULTS

IL-3 and IL-5 receptor expression in germ cells and spermatozoa

Expression of IL-3 and IL-5 receptor α -subunit mRNAs in human testicular cells was analysed by RT-PCR using primers specific for each α -subunit. An amplification product of approx. 510 bp was obtained from human testis RNA using primers for an internal region (size, 514 bp) of the IL-3 receptor α -subunit (Figure 1A, lane 2), while primers complementary to an internal region (size, 450 bp) of the IL-5 receptor α -subunit mRNA rendered an amplification product of 450 bp (Figure 1A, lane 3). The amplification product obtained from human testis RNA using primers specific for the β -subunit of the IL-3/IL-5 receptor was 570 bp long, matching the size of the amplification product predicted for the primer pair used (Figure 1A, lane 4). This was also the amplification product obtained when using RNA from haematopoietic cells that express IL-3, IL-5 and GM-CSF receptors (results not shown).

Immunoblotting of membrane proteins extracted from human spermatozoa revealed that, for the three antibodies, the reactivity was limited to one or two immunoreactive protein bands (Figure 1B). The IL-3 receptor α -subunit antibody reacted with two protein bands, a strongly immunoreactive protein band that migrated with an apparent molecular mass of 66 kDa, and a less prominent immunolabelled protein band with an apparent molecular mass of 38 kDa (Figure 1B). In contrast, the IL-5 receptor α -subunit antibody reacted with a single protein band with an apparent molecular mass of 61 kDa (Figure 1B). In a similar manner, the β -subunit antibody reacted with a single protein band with an apparent molecular mass of 105–110 kDa (Figure 1B). Confirming the specificity of the reaction, no immunoreactivity was observed when the antibodies were pre-adsorbed with the peptides used to elicit them (Figure 1B, lanes labelled +).

The presence and localization of protein products corresponding to the α - and β -subunits of the IL-3 and IL-5 receptors in adult human testis and in purified human spermatozoa was assessed in immunolocalization experiments with antibodies specific for each receptor subunit (Figures 2 and 3A). Positive immunoreactivity with the three antibodies (anti-IL-3 α -subunit, anti-IL-5 α -subunit and anti-IL-3/IL-5- β -subunit) was observed in the germ cells of the seminiferous tubules, and the immunoreactive material was associated with the plasma membrane and was also present intracellularly (Figures 2a–2f). There was a more intense immunostaining with the anti-(IL-3R α -subunit) antibody compared with that with the IL-5R α - and β -subunit-specific antibodies. There were clear differences in the intensity of immunoreaction of the cells with IL-3 and IL-5 α -subunit-specific antibodies compared with the anti- β -subunit antibody, suggesting a greater level of expression of both α -proteins. Strong signals were obtained in nuclei and nuclear periphery of germ cells, at different stages of proliferation and differentiation, from spermatogonia in the basal compartment to spermatids located near the lumen of the seminiferous tubules, with the most strongly labelled cells corresponding to spermatocytes. All cells labelled with both anti- α -subunit antibodies were also positive for the anti- β -subunit antibody,

Figure 1 Expression of IL-3 and IL-5 receptors in human germ cells and bovine spermatozoa

(A) RT-PCR of human IL-3 and IL-5 mRNAs. Total human testis RNA was subjected to RT-PCR using primers specific for the IL-3 and IL-5 α subunits or the β c subunit. PCR products corresponding to the IL-3 α (510 bp), IL-5 α (450 bp) and the β c (570 bp) subunits are shown in lanes 2, 3 and 4 respectively. The migration in the agarose gel of a series of DNA 100-mer size standards is shown in lane 1. (B, C) Identification of IL-3 and IL-5 receptors in spermatozoa. Membrane proteins isolated from human (B) and bovine (C) spermatozoa were fractionated by SDS/PAGE, transferred on to Immobilon membranes, and probed with anti-(IL-3 α -subunit), anti-(IL-5 α -subunit) and anti-(β c subunit) antibodies, followed by incubation with a secondary antibody coupled to horseradish peroxidase. Pre-adsorbed antibodies incubated with the peptides used to elicit them were used as negative controls (lanes labelled +). Sizes on the left in kDa indicate the migration of molecular-mass standards.

indicating that they express both receptor subunits simultaneously (Figures 2a–2f). Confirming the specificity of the reaction, no immunoreactive material was observed in control sections incubated with anti-(IL-3 receptor α -subunit) antibodies pre-adsorbed with the corresponding peptide antigen (Figures 2g and 2h), or when using pre-adsorbed IL-5 receptor α - and IL-3/IL-5 receptor β -subunit-specific antibodies (results not shown).

Human spermatozoa showed positive immunoreactivity with IL-3 and IL-5 receptor α - and β -subunit-specific antibodies, with intense immunolabelling observed mainly along the sperm tail (Figure 3A, panels a, c and e). With both anti- α -subunit antibodies, the strongest immunoreactivity was associated with the lower part of the head, the neck and the tail mid-piece, while with

Figure 2 Localization of IL-3 and IL-5 receptors in human testis

Testis sections were incubated with anti-IL-3 α -subunit (a, b), anti-IL-5 α -subunit (c, d) and anti- β c subunit (e, f) antibodies followed by incubation with a secondary antibody conjugated to horseradish peroxidase. Testis section incubated with anti-(IL-3 α -subunit) antibody in the presence of the peptide used to elicit it did not show a positive reaction (g, h). Arrows show positive staining in male germ cells. L, lumen of the seminiferous tubules. Scale bars, 20 μ m.

anti- β -subunit antibody, the immunoreactivity was associated to the tail. The specificity of the reaction was confirmed in experiments that showed absence of immunoreaction when pre-adsorbed antibodies were used (Figure 3A, panels b, d, and f).

Because of the difficulties associated with obtaining a highly purified and homogeneous population of human spermatozoa that could be used to dissect the functional status of the sperm cytokine receptors, we used bovine spermatozoa as an alternative cell model. A homogeneous population of bovine spermatozoa can be obtained in sufficient quantity to be used for *in vitro* glucose-uptake assays. Immunoblotting of membrane proteins extracted from bovine spermatozoa indicated the presence of protein bands reacting with both α - and β -subunit-specific antibodies (Figure 1C). The anti-(IL-3 receptor α -subunit) antibody reacted with two protein bands: a heavily immunolabelled protein band with an apparent molecular mass of 66 kDa and a less intensely labelled band with an apparent molecular mass of 38 kDa (Figure 1C). In a similar manner, two protein bands were recognized by the anti-(IL-5 receptor α -subunit) antibody: a labelled protein band with an apparent molecular mass of 61 kDa and a very intensely stained protein band of 30 kDa (Figure 1C). In contrast with the above, the anti- β -subunit antibody reacted with a single protein band with an apparent molecular mass of approx. 105–110 kDa (Figure 1C).

Figure 3 Immunolocalization of IL-3 and IL-5 receptors in human and bovine spermatozoa

Human (**A**) and bovine (**B**) spermatozoa were spread on to coated slides and probed with the anti-(IL-3 α -subunit) (a), anti-(IL-5 α -subunit) (c) and anti- β -subunit (e) antibodies, followed by incubation with a secondary antibody conjugated to horseradish peroxidase. No reactivity was observed with pre-adsorbed antibodies (b, d and f).

Confirming the results of the immunoblotting studies, bovine spermatozoa showed positive immunoreactivity with human IL-3 and IL-5 receptor α - and β -subunit-specific antibodies (Figure 3B, panels a, c and e). The immunoreactive material with both IL-3 and IL-5- α -subunit-specific antibodies was specifically localized in the sperm tail and the acrosomal region (Figure 3B, panels a and c). On the other hand, the anti- β -subunit antibody immunoreactive material was preferentially localized in the sperm tail (Figure 3B, panel e). For the three antibodies, the specificity of the reaction was confirmed in experiments that showed absence of immunoreaction when pre-adsorbed antibodies were used (Figure 3B, panels b, d and f).

Differential effects of IL-3 and IL-5 on hexose uptake in bovine spermatozoa and haematopoietic cells

The above results are consistent with the simultaneous expression of both α - and β -subunits of the IL-3 and IL-5 receptors in human and bovine spermatozoa, indicating that these cells express high-affinity receptors for both cytokines. To test whether the IL-3 and IL-5 receptors expressed in the sperm cells are functional, we examined the effect of IL-3 and IL-5 on the capacity of bovine spermatozoa to take up glucose (Figure 4A). We used GM-CSF as a positive control, which we have shown previously induces enhanced glucose uptake in human myeloid cells and in bovine sperm. As expected, GM-CSF induced a 2.5-fold dose-dependent increase in DOG uptake, with maximal uptake at 1 nM GM-CSF and 50% increase at 0.1 nM GM-CSF (Figure 4A). Similar to GM-CSF, IL-3 induced a dose-dependent increase in DOG uptake by bovine spermatozoa, with half-maximal activation observed at 100 pM and maximal activation at 1 nM IL-3 with a 2.5-fold increase in DOG uptake (Figure 4A). Concentrations of IL-3 up to

10 nM did not increase DOG uptake further (results not shown). In contrast, IL-5 failed to stimulate DOG uptake even at 1 nM (Figure 4A). TNF α , a cytokine that failed to induce enhanced hexose uptake in myeloid cells [9], did not stimulate hexose transport in bovine spermatozoa.

The above results in bovine spermatozoa indicated that IL-5 failed to induce enhanced hexose uptake under conditions at which both GM-CSF and IL-3 did so efficiently. We analysed this issue further by studying the effect of GM-CSF, IL-3 and IL-5 on hexose uptake in an eosinophilic subline of the myeloid leukaemia cells HL-60 that express high-affinity receptors for the three cytokines [16]. In HL-60 eosinophils, GM-CSF and IL-3 induced a time- and dose-dependent increase in DOG uptake (Figures 4B and 4C). Time-course analysis of the effect of GM-CSF revealed that the uptake rate increased for the first 30 min of incubation with 1 nM GM-CSF and remained constant for the next 30 min (Figure 4B). Dose-response analysis revealed that the uptake rate increased with increasing GM-CSF concentrations until it approached a plateau, with half-maximal activation at 0.1 nM GM-CSF and maximal activation (1.9-fold compared with basal levels) at 1 nM GM-CSF (Figure 4C). Similar results were obtained in IL-3-treated cells in terms of the time-course and the dose-dependent effects, although maximal activation was only 60% higher than the controls (Figures 4B and 4C). At 1 nM, IL-3 caused a maximal increase in the uptake rate after 30 min (Figure 4B), and half-maximal activation was observed at 0.3 nM IL-3, with maximal effect observed at 1 nM IL-3 (Figure 4C). In contrast with the above, no effect of IL-5 on hexose uptake was observed under similar experimental conditions, even at IL-5 concentrations (10 nM) that were at least 10-fold higher than the GM-CSF and IL-3 concentrations that caused maximal activation of DOG uptake (Figures 4B and 4C).

Figure 4 Effect of IL-3 and IL-5 on DOG uptake in bovine spermatozoa and in HL-60 eosinophils

(A) The DOG uptake by bovine spermatozoa in response to concentrations of IL-3 (●) and IL-5 (■) ranging from 0 to 1 nM at 30 min treatment time is shown. The results are displayed as nmol/min for DOG uptake and in cells treated with IL-3 and IL-5 relative to cells that were not treated. GM-CSF (○) and TNF α (▲) were used as positive and negative controls respectively. Results are the means $\pm$ S.D. of four samples. (B) Time-course of the effect of GM-CSF (○), IL-3 (●) and IL-5 (■) on DOG uptake by HL-60 eosinophils. Cells were treated with 1 nM GM-CSF, IL-3 and IL-5 for the time periods indicated, before measuring the uptake of DOG. Results are the mean of two experiments with three replicates each. (C) Dose-dependence of the effect of GM-CSF (○), IL-3 (●) and IL-5 (■) on DOG uptake by HL-60 eosinophils. Cells were incubated for 30 min in the presence of different concentrations of GM-CSF, IL-3 and IL-5, and uptake of DOG was measured afterwards. Results are the means $\pm$ S.D. of four samples, and represent one of three similar experiments.

As a control for assessing the biological activity of the three cytokines by an unrelated method, we tested the effect of GM-CSF, IL-3 and IL-5 on HL-60 cell proliferation. These studies revealed that HL-60 eosinophils responded to GM-CSF, IL-3 and IL-5 with increased cell proliferation and with a similar dose-dependence, although GM-CSF was the most effective inducer (results not shown). Thus the IL-5 receptor is able to transduce a proliferative signal in HL-60 eosinophils, but, similar to its lack of effect in bovine spermatozoa, is unable to stimulate enhanced hexose uptake.

DISCUSSION

In the present paper, we report a study addressing the issue of IL-3 and IL-5 receptor expression and function in male germ cells. Morikawa et al. [17] had previously demonstrated IL-3 receptor expression in mouse testicular Leydig cells, but failed to detect expression of the GM-CSF or the IL-5 receptor α -subunit, and no expression of the three cytokine receptors was found in testicular germ cells [17]. In the present study, the presence of IL-3 and IL-5 receptors in male germ cells of human and bovine origin was demonstrated by the unrelated methods of RT-PCR, immunolocalization and immunoblotting, and functional assays.

The results of the RT-PCR experiments indicated that human male germinal cells express mRNAs for the α - and β -subunits of the IL-3 and IL-5 receptors. Moreover, the immunolocalization experiments confirmed the presence of the α - and β -proteins in all germ cells, from spermatogonia to spermatids. The immunolocalization experiments revealed that there was expression of the receptors not only at the plasma membrane, but there was also significant intracellular expression, including a clear pattern of nuclear staining. In the absence of results from high-resolution localization studies coupled with the use of markers for specific subcellular structures, the significance of the nuclear staining pattern for the α -subunit of the IL-3 and IL-5 receptors is unknown.

The results also revealed expression of IL-3 and IL-5 receptors in spermatozoa of human and bovine origin. Although there was some variability in the intensity of staining in the different areas of the sperm membrane, the immunolocalization experiments, using anti- α - and β -subunit antibodies, indicated colocalization of the α - and β -subunits of the IL-3 and IL-5 receptors along the sperm tail, which is compatible with the formation of high-affinity IL-3 and IL-5 receptor $\alpha\beta$ -heterocomplexes. Moreover, the results of the immunoblotting experiments support the notion that the human and bovine spermatozoa IL-3 and IL-5 receptors have a subunit structure similar to the corresponding receptors present in haematopoietic cells for which the α - and β -subunits have been characterized in detail. In haematopoietic cells, both the α - and β -subunits of the mature IL-3 and IL-5 receptors are glycoproteins that migrate in SDS/polyacrylamide gels at a higher molecular size than that expected from the predicted protein encoded by the respective genes. Thus the high-molecular-mass proteins detected by immunoblotting with anti- α - and β -subunit antibodies in human and bovine spermatozoa probably correspond to the fully glycosylated α - and β -subunits of the IL-3 and IL-5 receptors. We have described similar results for the GM-CSF receptor in male germ cells [12].

Biological response analyses confirmed the presence of functionally active IL-3 receptors in bovine spermatozoa. Human IL-3 induced increased uptake of DOG in a dose-dependent manner that closely matched the effect of this cytokine in human haematopoietic cells [11]. The effect of IL-3 on DOG uptake by sperm cells was clearly evident at low concentrations, around 100 pM, and uptake increased further at concentrations of IL-3 in the

nanomolar range, an observation that is compatible with the presence of high-affinity IL-3 receptors in bovine spermatozoa and is supported further by the immunolocalization experiments that indicate co-localization of the IL-3 receptor α - and β -subunits on the sperm tail.

In contrast with the above, IL-5 failed to induce increased hexose uptake in bovine spermatozoa, cells in which the immunolocalization and immunoblotting experiments revealed expression of IL-5 receptors and co-localization of the α - and β -subunits in the sperm tail. Although there are reports indicating that GM-CSF and IL-3 induce increased uptake of hexoses in haematopoietic cells [6,7,9,11], to our knowledge there are no reports indicating that IL-5 may induce a similar effect. The lack of effect of IL-5 on hexose uptake was confirmed in HL-60 eosinophils that responded to IL-5 with increased proliferation, but showed no increase in hexose uptake. In contrast, in these cells, GM-CSF and IL-3 induced an increase in both cell proliferation and hexose uptake.

In human cells, the myeloid colony-stimulating factors stimulate increased glucose uptake in target cells, presumably to provide increased metabolic fuel for heightened cellular activity. The present study reports increased glucose uptake by bovine spermatozoa stimulated with human IL-3. Although the role of the haematopoietic growth factors and their receptors in normal testicular and sperm physiology is currently unknown, our results suggest that IL-3 and GM-CSF may have a functional role in the normal physiology of the male germ cells. Increased glucose uptake may be related to an increased use of sugars as metabolic fuels. Human and bovine spermatozoa express several members of the family of facilitative hexose transporters and efficiently transport the energy-producing sugars glucose and fructose, and the oxidized form of vitamin C [15,18].

Our results indicating no effect of IL-5 on bull sperm hexose uptake makes it difficult to propose a specific role for IL-5 and its receptor in the physiology of the germ cells. In contrast, in haematopoietic cells, IL-5 shows biological effects that overlap with those of IL-3 and GM-CSF. As for the structural basis of this behaviour, although there is no significant amino acid sequence similarity among these three cytokines, they exhibit a number of structural-functional similarities. IL-3, IL-5 and GM-CSF are cytokines composed of four α -helices, and their gross tertiary structures are similar; binding of IL-3 to its receptor is competed by GM-CSF and vice versa, and, likewise, IL-5 binding to the receptor is also competed with either IL-3 or GM-CSF [19]. Many cells of the haematopoietic lineage that express either IL-3 or IL-5 receptors also express GM-CSF. Previous studies have suggested that IL-3 and IL-5 are able to activate pre-formed GM-CSF receptors, thus raising the possibility that the biological functions of IL-3 and IL-5 are mediated in part by signalling through the GM-CSF receptor. A further possibility is that the pre-formed $\alpha\beta$ -GM-CSF receptor complex may act to potentiate the effects of IL-3, IL-5 and GM-CSF by reducing the need for multiple ligand-induced heterodimerization events [20]. Because the IL-3 and GM-CSF high-affinity $\alpha\beta$ -receptor heterocomplexes share one component (the β -subunit), the cross-competition of binding between GM-CSF and IL-3 is likely to occur by two different α -subunits competing for a limited number of β -subunits [21].

In summary, we have demonstrated the presence of the IL-3 and IL-5 receptors in human and in bovine male germ cells, and shown that bovine spermatozoa express functional IL-3 receptors that signal for increased glucose uptake. On the other hand, no increased hexose uptake was observed in the presence of IL-5 in bovine spermatozoa as well as in haematopoietic cells. These results establish that these cytokines, typical haematopoietic growth

factors, may have an important role in the physiology of male germ cells, and that differential signalling for increased hexose uptake by heteromeric high-affinity IL-3 and IL-5 receptors in mammalian spermatozoa is a property that depends on the identity of the α -subunit forming part of the $\alpha\beta$ -heterocomplex and is not a property specific to the germ cells. Clearly, cross-talk between different receptors has the potential to increase the signalling repertoire for a receptor family, and may account for some of the diverse biological functions and differential signalling attributed to GM-CSF, IL-3 and IL-5.

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