Differential expression of HOX genes upon activation of leukocyte sub-populations

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Abstract The HOX genes are key determinants of cellular identity both in early development and in the renewal and differentiation of adult blood cells. Although a number of studies have examined the expression of individual HOX genes in defined blood cell lineages, we have undertaken a comprehensive analysis of HOX gene expression in resting and activated lymphocytic and monocytic subpopulations. This has revealed distinct patterns of expression between different cell types and resting and activated states. (Main category A: Erythrocytes, Leukocytes and Hematopoiesis, subcategory: 8: Lymphocytes).

Keywords HOX · T-lymphocytes · B-lymphocytes · Monocytes · Activation

1 Introduction

The HOX genes are a family of homeodomain-containing transcription factors that define specific positional identities, both in vertebrate and invertebrate embryos [4]. They are also involved in the regulation of haemopoiesis (reviewed by [13]), and blocking HOX gene function by the use of antisense oligos or gene knock out perturbs a number of different haemopoietic events. Thus for example myeloid, erythroid and lymphoid haemopoiesis are all defective in HoxA9 knock out mice [8], and the antisense ablation of HoxB5, HoxB6, HoxB7 [15], or HoxA5 [5] blocks erythroid differentiation. Forced expression of HOXA10 in CD34+ progenitor cells purified from cord blood results in a significant reduction in the number of B cells and natural killer cells but a marked increase in monocytes [14]. There are also examples of specific regulatory functions for HOX genes, including the modulation of globin transcription by HOXB6 [12]. In addition, HOX genes can also act to regulate the proliferation of haemopoietic stem cells (HSCs). Hence HOXB4 is a strong, positive regulator of HSC self renewal (reviewed by [10]), and an estrogen-dependant version of HOXB8 can be used to drive the production of neutrophils and macrophages from their respective progenitor cells [16].

Despite these studies, relatively little is still known about the expression of HOX genes in the mature blood lineages (reviewed by [6]). In order to address this we purified different cell populations from human peripheral blood on the basis of their expression of the surface markers CD4, CD8, CD14 and CD19 that are present on T-lymphocytes (CD4 and CD8), monocytes, and B-lymphocytes respectively. HOX expression was compared in ‘resting’ cells and cells that had been activated by challenging with specific antigenic stimuli—antibodies against CD2, CD3 and CD28 for CD4+ and CD8+ cells, Lipopolysaccharide for CD14+ cells and anti-IgG for CD19+ cells. Each population of cells underwent proliferation in response to this treatment, as measured by [3H]thymidine incorporation (Fig. 1). RNA was extracted from each cell type and the expression of each of the 39 HOX genes was assed by quantitative PCR (QPCR), relative to the amount of Beta-actin transcript present (Fig. 2).

The highest level of expression is generally shown by the HOXA and HOXC genes, with HOXD genes generally
exhibiting a tenfold lower expression level and HOXB genes a 100 fold lower expression level, a feature that is also true for the other cell populations examined. For all of the HOX genes expressed in CD4+ cells there is a significant reduction in HOX expression upon activation. This is particularly striking for the most posterior genes of the HOXA, HOXC and HOXD clusters. A similar pattern is also observed in CD8+ cells, although there is a general reduction in HOX expression with every HOX gene except HOXC10 being present at lower levels in these cells.

There are a number of notable differences between the HOX expression profile of CD14+ cells and the CD4+/CD8+ populations. HOXC4, HOXC6 and HOXC8 are not expressed. The most striking difference though is the change of HOX expression upon activation—in contrast to CD4+/CD8+ cells, CD14+ cells show a large increase in HOXA2, HOXB13, HOXC10, HOXD13, HOXD1 and HOXD9. Other HOX genes such as HOXB4 and HOXA10 show no significant change in expression upon activation, which again contrasts with the response of CD4+/CD8+ cells.

CD19+ cells show a broadly similar pattern of HOX expression to CD14+ cells prior to activation, but have a distinct response to activation. Like CD4+/CD8+ cells, CD19+ cells exhibit a large reduction in expression of most HOX genes with the exception of HOXD10, HOXD11 and HOXD12 that show a very strong increase in expression upon activation.

To our knowledge this is the first comprehensive analysis of HOX gene expression in these leukocyte populations. Taking the data as a whole, each population shows a unique pattern of HOX expression that may define cellular identity. Generally the expression levels are in agreement with those previously reported for individual HOX genes in other studies [6, 7, 9]. However the changes of HOX gene expression upon cellular activation have not previously been reported. The magnitude of these changes is striking, with the activated T cell subsets exhibiting an almost complete loss of expression of many HOX genes especially the more posterior members of the groups.

Fig. 1 Proliferation of activated lymphocyte populations. Proliferation was measured by [3H]thymidine incorporation 4 h after activation. Values shown are the fold increase in incorporation compared to resting cells. Error bars: SEM from triplicate experiments.

Fig. 2 HOX gene expression in resting (a) and activated (b) after 6 h, (c) after 24 h lymphocytic and monocytic populations. Expression was assessed by quantitative PCR and is shown as a relative value to the number of beta-actin transcripts (‘Relative expression’). HOX genes are abbreviated to show only their family and paralogue position, hence HOXA2 is shown as ‘A2’. HOX genes for which no expression was detected are not shown in the figure. Each value shown is the mean from three experiments. Significant changes in expression levels (p < 0.05) 6 and 24 h after activation are marked (*). Error bars: SEM.
(HOXA10, HOXC13, and HOXD12). Conversely, activated monocytic and B-lymphocyte cells show a large increase in expression of the same genes. Given the key regulatory roles of HOX genes it is likely that these changes in expression facilitate the cellular changes associated with activation. One of these is increased proliferation, a response that is known to be mediated by HOX genes in a number of hematopoietic lineages (reviewed by [1]). Indeed the antisense mediated ablation of HOXB2 or HOXB4 in T-lymphocytes was previously shown to block the proliferation of activated cells (Care et al. 1995). Furthermore HOXA10 deficiency causes a severe immunological disturbance in uterine tissues characterised by rapid T cell proliferation and a failure of progesterone-mediated immunosuppression [17]. The HOX genes are also likely to regulate lineage-specific transcription upon
activation, and in this context the contrasting expression levels of these genes presumably reflect the dramatically different response of each cell type.

2 Materials and methods

CD4+, CD8+, CD14+ and CD19+ cells were isolated using the Dynabead system (Invitrogen) and cultured as previously described [2]. Quantitative PCR was performed as previously described [11]. [3H]thymidine incorporation was evaluated by a standard procedure. Briefly, 2 µCi of [3H]thymidine (20 Ci/mmol) was added for 4 h to each well. Cells were then recovered, washed, and processed for the determination of TCA precipitable radioactivity. Each evaluation was performed in triplicate. T cell activation was achieved using anti-CD2, anti-CD3 and anti-CD28 antibodies as supplied in the T cell activation/expansion kit (Miltenyi Biotec).

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