ImmunoTools IT-Box-139 Award 2013

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New strategies for mesenchymal stromal cell recruitment to bone injury sites

Balancing the high prevalence of bone diseases in worldwide population and the therapeutic strategies currently available to treat them, it is clear that new strategies for bone repair/regeneration need to be developed. Recruitment of mesenchymal stem cells (MSC), the progenitors of osteoblasts, to bone injury sites may be the starting point for some of these strategies. Immune cells naturally produce a plethora of chemotactic signals, some of which may be directed to specific cell types. Thus, our aim is to investigate cell-directed factors able to modulate MSC migration. To achieve our purpose, immune cells in different inflammatory conditions will be characterized and used to perform MSC migration assays *in vitro.*

Immune cells will be derived from buffy coats of healthy human blood donors, and the identity of the cell populations isolated will be routinely tested by flow cytometry for characteristic lineage markers, such as CD45, HLA-DR, CD1a, CD11c, CD14, CD33, CD56, CD3, CD4, CD8, CD19, CD20 and CD66b. The different immune cell populations isolated will be cultured in conditions simulating inflammatory environments (for instance, adding TNF-α (ImmunoTools)), followed by an evaluation of their maturation/activation status by conventional or imaging flow cytometry. Their morphology and the cell distribution of activation markers, as CD25, CD40, CD80, CD83, CD86, CD54, CD22 and CD71 will be then analysed. Also, AnnexinV combined with propidium iodide will be used to determine if apoptosis is being induced in our assays.

On the other hand, to be sure of working with MSC, their phenotype has to be characterized, including for the presence of the surface markers CD105, CD73 and CD90 with the concomitant absence of the lineage markers CD45, CD19, CD34, CD14 and HLA-DR, which will be done by flow cytometry.

Changes on cell migration will be evaluated monitoring the presence and distribution of molecules involved in cell adhesion, like CD11a/CD18, CD29, CD31, CD44, CD50, CD54, CD61 and CD62L.

In this way, throughout the whole project different methodologies will be applied for the immunodetection of CD antigens recognized by most of the antibodies of the *IT-Box-139*, including the cutting-edge technique of imaging flow cytometry. In addition,

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since many of the antibodies included in the *IT-Box-139* are available conjugated with different fluorochromes, it allows for multiple combinations with other ImmunoTools antibodies that we already routinely use, simplifying the assays and saving time and scarce samples. Taken together, the use of *IT-Box-139* in this project will be very useful for the majority of the assays, confirming the versatility of ImmunoTools antibodies and demonstrating their application in areas currently under intense research.

ImmunoTools *IT-Box-139.2* for **Andreia Machado da Silva** includes 100 antibodies

FITC - conjugated anti-human CD1a, CD3, CD4, CD5, CD6, CD7, CD8, CD14, CD15, CD16, CD19, CD21, CD25, CD29, CD35, CD36, CD41a, CD42b, CD45, CD45RA, CD45RB, CD45RO, CD49d, CD53, CD57, CD61, CD63, CD80, CD86, HLA-DR, IL-6, Control-IgG1, Control-IgG2a, Control-IgG2b, Annexin V

PE - conjugated anti-human CD3, CD4, CD8, CD11b, CD15, CD14, CD18, CD19, CD20, CD21, CD22, CD31, CD33, CD38, CD40, CD45, CD45RB, CD50, CD52, CD56, CD58, CD62p, CD72, CD95, CD105, CD147, CD177, CD235a, HLA-ABC, IL-6, Control-IgG1, Control-IgG2a, Control-IgG2b, Annexin V

PE/Dy647 -tandem conjugated anti-human CD3, CD4, CD8, CD14, CD19, CD20, CD25, CD54

APC -conjugated anti-human CD2, CD3, CD4, CD8, CD10, CD11a, CD11c, CD14, CD16, CD27, CD37, CD42b, CD44, CD45, CD59, CD62L, CD69, CD71, IL-6, Control-IgG1, Control-IgG2a, Control-IgG2b, Annexin V

DETAILS

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