PHYLOGENETIC ANALYSIS BY MOLECULAR SEQUENCE OF VARIOUS HUMAN INTERLEUKINS

ABSTRACT

Due to the importance of interleukins in the immune response, in cell differentiation, and their potential use to treat autoimmune diseases and tumors, in this article we decided to perform a phylogenetic classification according to the molecular sequence of several interleukins.

From the Uniprot website, the molecular sequences of 16 human interleukins were downloaded in FASTA format, using the free software mega11. Using a statistical model of maximum likelihood, the phylogenetic tree was built; subsequently, the constants were incorporated to calibrate the time tree marker.

Our results show that in the upper paleolithic, the first interleukins of homo sapiens sapiens were profiled. The evolutionary history of 8 interleukins probably occurred in the Mesolithic period. In the neolithic period, already with the discovery of agriculture, 6 interleukins were developed.

This paper presents an overview of the most likely evolutionary history of the interleukin lineage.

1. INTRODUCTION

Interleukins (IL's) are proteins that act as communication mediators between cells of the immune system and have an important role in the body's immune response (1). IL's are produced by a variety of cells, such as T, B lymphocytes; natural killer (NK) cells, macrophages and dendritic cells (2).

So far, more than 30 different types of IL's have been discovered, each plays a role in regulating the immune response. IL’s have been classified into two main categories: pro-inflammatory and anti-inflammatory (3).

IL-1, IL-6 and IL-8 promote inflammation and immune response in the body. They are generated in response to infections, injuries or diseases and are considered responsible for the activation of immune cells, such as neutrophils and macrophages (3). Anti-inflammatory IL’s, such as IL-4, Il-10 and IL-13, inhibit inflammation, immune response and limit excessive tissue damage (3). A table with the main activity of each IL is presented (3):

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IL	Main activity
IL-1	proinflammatory
IL-2	proinflammatory, promotes T cell proliferation
IL-3	stimulates growth and differentiation of hematopoietic cells
IL-4	anti-inflammatory, activation, proliferation and differentiation of B lymphocytes
IL-5	anti-inflammatory, stimulator of the activation, growth and differentiation of B lymphocytes
IL-6	proinflammatory
IL-7	mitogenic, stimulates the development of B and T lymphocyte precursor cells
IL-8	proinflammatory, chemotactic for leukocytes
IL-9	mitogenic, induces T cell proliferation
IL-10	anti-inflammatory
IL-12	proinflammatory
IL-13	anti-inflammatory
IL-15	proinflammatory, induces T cell proliferation
IL-16	proinflammatory
IL-17	proinflammatory
IL-18	proinflammatory

Table 1. Properties of IL's

Due to the importance of IL's in the immune response, in cell differentiation and its potential use to treat autoimmune diseases and tumors; we decided to perform a phylogenetic classification through the molecular sequence of several IL's in order to identify possible evolutionary relationships between them.

Phylogenetic analysis seeks to reconstruct the evolutionary history of living beings from genetic, morphological or molecular characteristics. The evolutionary relationships between different organisms are represented by a phylogenetic tree (4).

A phylogenetic tree is a diagram that represents the possible evolutionary relationships between organisms. It is constructed by comparing the characteristics that organisms share. It is important to mention that phylogenetic trees are suggestions, not definitive facts (4).

There are several methods for constructing phylogenetic trees from molecular sequences. In general, they can be classified into distance, parsimony, and probability methods (4).
In this article, probability methods were considered for the construction of the phylogenetic tree, since they rely on maximum likelihood estimators to explain the compared characteristics (5).

2. MATERIALS AND METHODS

From the UNIPROT website (6), the molecular sequences of the following IL's were downloaded in FASTA format:

IL1B_HUMAN Interleukin-1 beta
IL2_HUMAN Interleukin-2
IL3_HUMAN Interleukin-3
IL4_HUMAN Interleukin-4
IL5_HUMAN Interleukin-5
IL6_HUMAN Interleukin-6
IL7_HUMAN Interleukin-7
IL8_HUMAN Interleukin-8
IL9_HUMAN Interleukin-9
IL10_HUMAN Interleukin-10
IL12B_HUMAN Interleukin-12 beta
IL13_HUMAN Interleukin-13

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IL15_HUMAN Interleukin-15
IL16_HUMAN Pro-interleukin-16
IL17F_HUMAN Interleukin-17F
IL18_HUMAN Interleukin-18

With the free software program BIOEDIT (7), the file with the sequences of the IL's with extension *.txt, was transformed into a file with extension *.fas.

Sequential alignment was performed using the free software MEGA11 (8), using the ClustalW algorithm. Subsequently, with the same MEGA11 program, the best statistical model of maximum likelihood was sought to build the phylogenetic tree for the proposed IL's.

With the statistical model of maximum likelihood found, the phylogenetic tree was constructed. Internal constraint nodes were used. The calibration constants of the time marker were:
!MRCA='sp|P29460|IL12B HU... GN IL12B PE 1 SV 1-sp|P05231|IL6 HUMA...06 GN IL6 PE 1 SV 1' TaxonA='sp|P29460|IL12B HUMAN Interleukin-12 subunit beta OS Homo sapiens OX 9606 GN IL12B PE 1 SV 1' TaxonB='sp|P05231|IL6 HUMAN Interleukin-6 OS Homo sapiens OX 9606 GN IL6 PE 1 SV 1' Distribution=normal mean=100.00000000 stddev=0.15000000 calibrationName='sp|P29460|IL12B HU... GN IL12B PE 1 SV 1-sp|P05231|IL6 HUMA...06 GN IL6 PE 1 SV 1-split';

The MEGA11 program estimates divergence times for all branch points in a phylogenetic tree, using the RelTime method (5).

There is a relative consensus among the various archaeological and anthropological studies, that modern experts called Homo sapiens sapiens, appeared 120,000-100,000 years ago (9). Therefore, the time scale was set at 100,000 years.

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3. RESULTS AND DISCUSSION

Once the sequential alignment was carried out, the MEGA11 program sought the best maximum likelihood model to build the phylogenetic tree.

The result corresponded to the model developed by Whelan and Goldman (10), called WAG+G+F, which uses a discrete gamma distribution to model differences in evolutionary rate between estimated sites. The value was 6.3482, generated by MEGA11 software (5).

Figure 1 shows the phylogenetic tree developed by the WAG+G+F method.

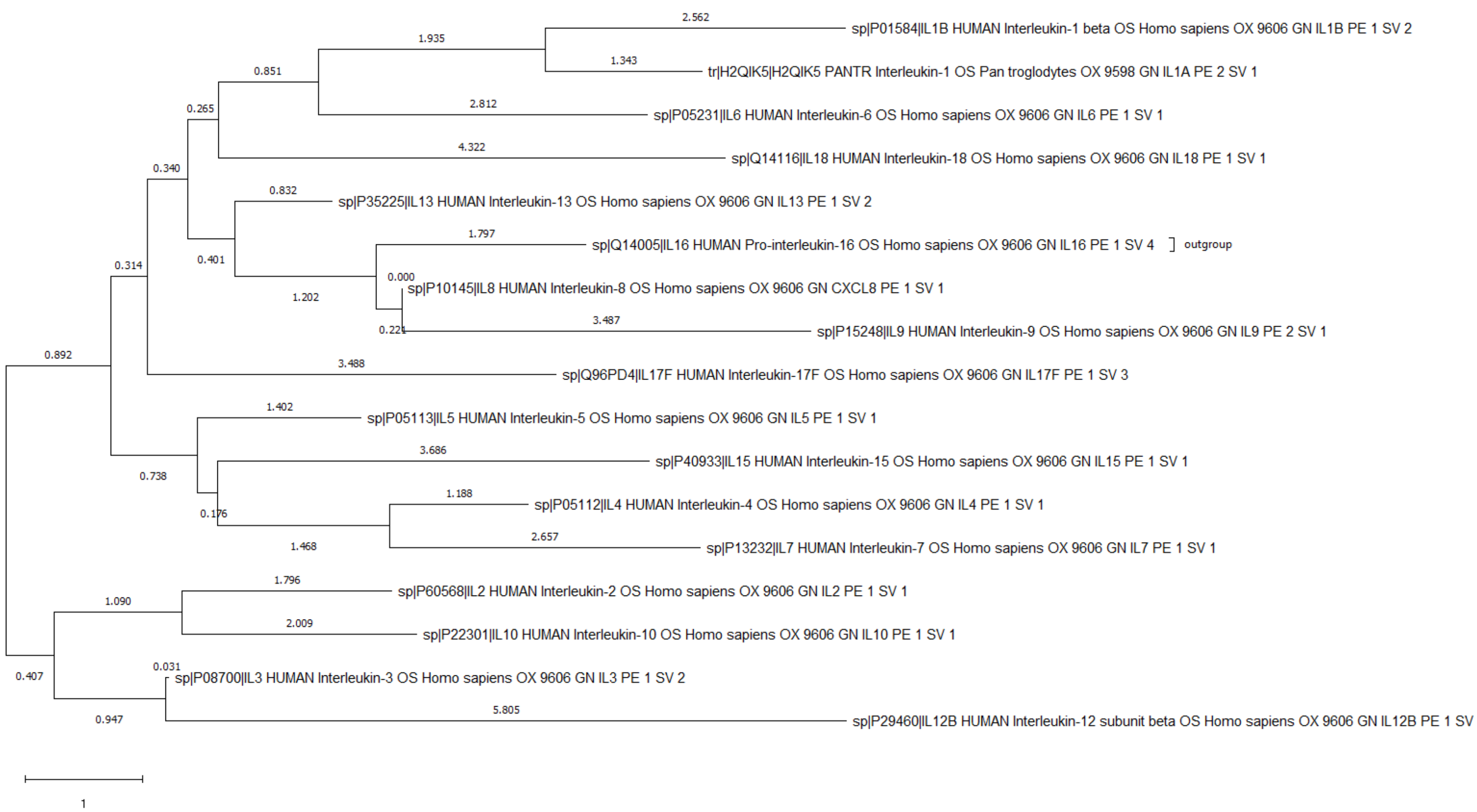

Figure 1. Phylogenetic tree developed by the method WAG+G+F

Once the phylogenetic tree was constructed, using the WAG+G+F model, the criteria for estimating the divergence times at the branching points were defined. The phylogenetic tree with the divergence times at the branching points is presented in Figure 2.

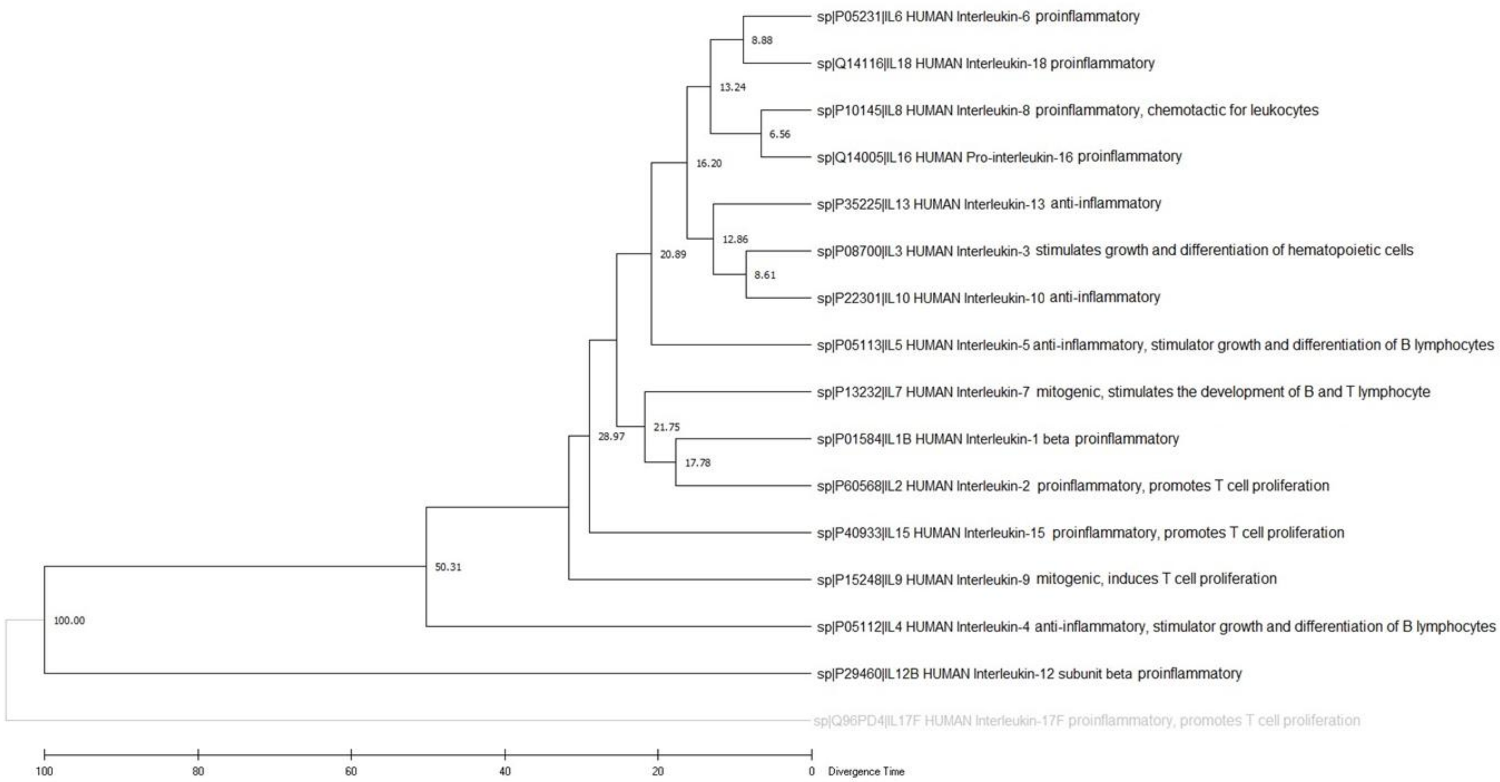

Figure 2. Phylogenetic tree with divergence times at branch points

In **Error! Reference source not found.**a cladogram is presented with the timeline and the Upper Paleolithic, Mesolithic and Neolithic periods, with the dates accepted by most scientists (11).

According to the results of the cladogram in **Error! Reference source not found.**, an overview of the most likely evolutionary history of the lineage of the IL's is presented.

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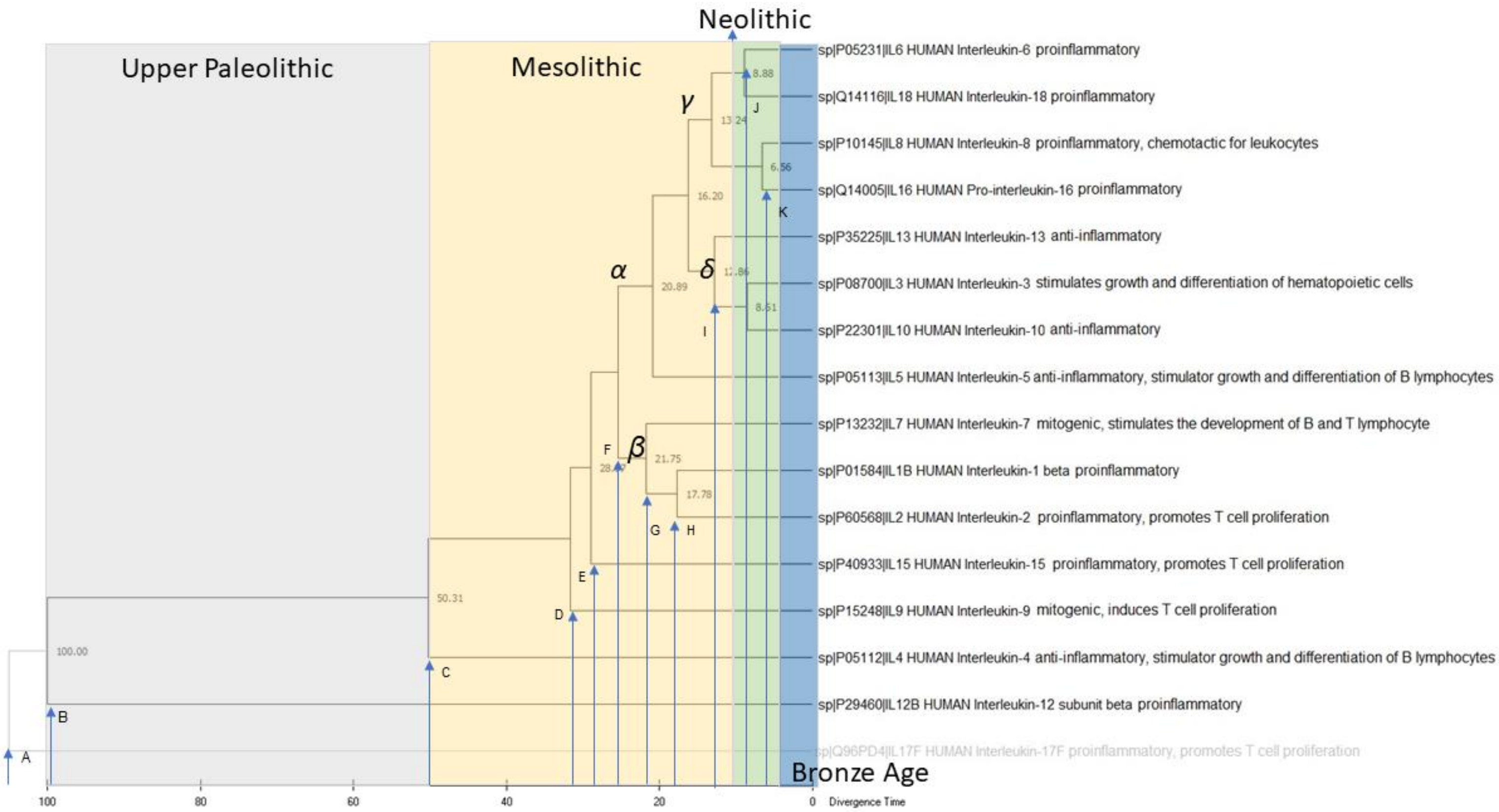

Figure 3. Phylogenetic tree with the Upper Paleolithic, Mesolithic and Neolithic periods

The first branching, called in this article A, possibly occurred at the end of the Middle Paleolithic, where IL 17 is separated from a common ancestor and remains until today, with a proinflammatory activity.

Because inflammation is considered a protective response to injury where immune system cells attack and destroy invading bacteria, parasites, or viruses and eliminate the tissue destruction they cause, and initiate the repair process (3): it stands to reason that one of the first ILs that evolved with primitive Middle Paleolithic human was a pro-inflammatory IL, due to their nomadic lifestyle, where food depended on hunting animals, fishing, and gathering plants, wild fruits, and roots. Archaeological studies show that humankind faced all kinds of dangers, and it is logical to assume that in many cases his health was affected (11).

According to our results, at the beginning of the Upper Paleolithic, a new branching possibly occurred in the evolutionary line of the IL's (see stage **Error! Reference source not found.**); generating 2 clades, the first generated a common ancestor for other IL's that would later branch and the second clade gave rise to IL12 with proinflammatory activity. According to some studies, there was a genetic exchange between Homo sapiens and Homo neanderthalensis during the Upper Paleolithic. It is not possible to know the impact that this event had on the development and evolution of the various IL's (12). During this period, humankind was still nomadic, and it suffered diseases and pain, which is consistent with the appearance of IL2 with proinflammatory activity.

The next branching, (see stage **Error! Reference source not found.**occurred at the end of the Upper Paleolithic and early Mesolithic, approximately 50,000 years ago. According to archaeological studies, it is the time when the oldest human settlements have been detected, and possibly the discovery of the bow and arrow took place as well (13).

According to our results, another branching occurred approximately 50,000 years ago, generating two clades. One of them formed a common ancestor for other IL's that would later branch out; the other clade gave rise to IL4, with anti-inflammatory and stimulating activity of activation, proliferation and differentiation of B lymphocytes. The appearance of this anti-inflammatory IL occurs with an important change in the habits of humans at the beginning of the Mesolithic, where humans used settlements in summer and shelters during the winter (14)

According to our results, approximately 30,000 years ago an additional branching occurred, see stage D-Figure **Error! Reference source not found.**. At that time, during the Mesolithic, the first known cave paintings appeared (15) along with the possible domestication of dogs (16). The branching generated two clades, one of them formed a common ancestor for other IL's that will later branch out; the other clade gave rise to IL 9, with mitogenic activity and that stimulates the development B cells precursor, which denotes a reinforcement of the immune system towards the middle of the Mesolithic period, where important dietary changes occurred with abundant and relatively safe diets (14).

It is interesting to mention that the appearance of this IL, with the capability to strengthen the immune system, coincides with the first findings of anthropomorphic figures that identify the first sorcerers and shamans, as well as cave paintings, where hunting scenes, dances and religious magical healing rituals are represented (14).

A next branching happened approximately 29,000 years ago, stage **Error! Reference source not found.**. The common ancestor of the IL's generated 2 clades, one of them formed a common ancestor for other IL's that will later branch; the other clade gave rise to IL15 with marked proinflammatory activity and responsible for promoting the proliferation of T cells. Archaeological evidence shows the appearance of the first ovens found during this time as well, which may have some relationship, but further studies are required to confirm the claim (17).

According to our results, the next branching occurred 28,900 years ago in the mid-Mesolithic, stage **Error! Reference source not found.**. The first clay figures known to history have been found to be in a similar date, such as the Venus of Dolni Věstonice (18). The use of fibers to make baby carriers, clothes, bags, baskets and nets was also developed during this period (19). From this branch, 2 important clades were generated, which for the purposes of this article will be called α and β, from where the remaining IL's shown descend. The evolutionary lineage of these 2 IL's lasted around 7000 years.

According to our results, approximately 21,700 years ago, the appearance of IL-7, responsible for mitogenic activity and stimulating the development of T and B lymphocytes, occurred in clade β, see stage G-Figure**Error! Reference source not found.**. In clade α, IL-5 was generated, with anti-inflammatory activity and responsible for stimulating proliferation and differentiation of B lymphocytes. The appearance of these IL's coincides with changes in human behavior, since at that time the oldest permanent human settlements appeared (20).

With the appearance of these two new IL's, one anti-inflammatory and the other stimulating the growth and differentiation of B lymphocytes, the oldest migratory waves were also identified in the American continent (19).

According to our results, approximately 18,000 years ago, from the common ancestor of clade β, see stage H-Figure**Error! Reference source not found.**IL's 1 beta and IL 2 appear, both with proinflammatory activity. At that time, the Earth lived the hardest moments of the last Ice Age (11).

Our results show that before the end of the Mesolithic, see stage **Error! Reference source not found.**16,000 years ago, the common ancestor of clade α, generated two evolutionary branches,

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which for the objectives of this article will be called γ (gamma) and δ (delta). One of the branches of the clade δ gave rise to IL 13 with anti-inflammatory activity. It is interesting to mention that the appearance of this IL with anti-inflammatory capacity coincides with the increase in findings of cave paintings of anthropomorphic beings, half men and half animals, possibly sorcerers and shamans, present in scenes of hunting, dance and religious magical healing rituals (21), which would indicate further changes to human lifestyle.

According to our results, one of the branches of the clade δ, approximately 12,800 years ago, at the end of the Mesolithic, would give rise to IL 13, with anti-inflammatory activity. At that time the late glacial maximum and end of the last glacial period occur; Climate warms and glaciers retreated (22).

The evolutionary lineage of the other branch of the clade δ, lasted for about 4,000 years, until the Neolithic, about 8,500 years ago. This branch would generate two IL's: IL 3, with important function in the differentiation of hematopoietic cells, and IL10 with anti-inflammatory capacity. During this time, in Mesopotamia, today Iraq, the first crops of barley and wheat appear (23).

According to our results, the evolutionary lineage of the common ancestor of the clade γ, lasted around 5,000 years and is until the Neolithic, 6,500 years ago, when it would generate two IL's, IL 8 with proinflammatory and chemotactic activity for leukocytes, and IL 16, with proinflammatory activity. At that time, the last Neolithic civilizations disappear, the invention of the wheel and the spread of protowriting occurs (24).

With certainty, the man of the Lower Paleolithic and Middle Paleolithic, had proteins similar to the current IL's, but it is not possible to identify them, because the lineage of the known species of the genus Homo, disappeared or mixed in different evolutionary phases. Possibly, these proto-IL's, gradually ceased to be synthesized, in response to the different environmental conditions and lifestyles that human ancestors experienced in their evolution and adaptation, gradually giving way to the current IL's.

4. Conclusion

Our results show that in the Upper Paleolithic, the first interleukins of Homo sapiens sapiens were profiled. The evolutionary history of 8 interleukins probably occurred in the Mesolithic period. In the Neolithic period, already with the discovery of agriculture, 6 interleukins were developed.

Our results show that the appearance of different IL's throughout the history of humanity, from the Paleolithic to the Mesolithic, coincides with climatic changes, with changes in the diet and / or lifestyle of humankind. In addition, some archaeological findings could be relevant to understand how human evolution influenced the development of IL's, such as the genetic exchange between Homo sapiens and Homo neanderthalensis.

In this article, the appearance of pro-inflammatory IL's coincides with times when man faced all kinds of dangers, and it is logical to assume that in many cases his health was affected. In these cases, proinflammatory ILs promoted the protective response to injuries and infections, initiating the process of tissue repair.
It is interesting to note that our data correlates the appearance of anti-inflammatory IL's with the appearance of important spiritual habits and beliefs of humans, such as the emergence of shamans, healers and magical-religious rituals for the restoration of health.

With certainty, the man of the Lower Paleolithic and Middle Paleolithic, had proteins similar to the current IL's, but it is not possible to identify them, because the lineage of the known species of the genus Homo, disappeared or mixed in different evolutionary phases. Possibly, this proto-IL's, gradually ceased to be synthesized in response to the different environmental conditions and lifestyles that human ancestors experienced in their evolution and adaptation, gradually giving way to the current IL's.

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ABBREVIATIONS

L's Interleukins
IL Interleukin

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