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INNATE LYMPHOID CELLS. NEW PLAYERS IN TISSUE HOMEOSTASIS AND INFLAMMATORY RESPONSES

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Abstract In recent times, our understanding of the role of the immune system in different physiopathological situations has increased markedly. A new set of cells, generically known as innate lymphoid cells (ILC), has been discovered in the lymphoid compartment. Five ILC subsets can be recognized according to phenotypic and functional similarities with different subpopulations of T lymphocytes. Unlike T and B lymphocytes, ILC do not express antigen receptors nor undergo selection and clonal expansion upon activation. Instead, they respond rapidly to cytokines and danger signals in infected or inflamed tissues, producing cytokines that direct the immune response toward a type suitable for controlling the initial insult. In addition, ILC establish a crosstalk with other cells of the microenvironment that contributes to the maintenance and restoration of tissue homeostasis. Although many evidences on ILC were obtained from animal models, solid data confirm their existence in humans and their role in various inflammatory disorders. In this article, we address new knowledge on ILC, particularly on their role in the homeostasis of the immune system and in various inflammatory pathologies, in order to present new actors regulating immunity and immunopathology and affecting human health.

Key words: innate immunity, cytokines, transcription factors, inflammation, homeostasis

Resumen ***Las células linfoides innatas. Los nuevos actores en la homeostasis tisular y las respuestas inflamatorias.*** En tiempos recientes, nuestra comprensión del rol del sistema inmune en diferentes situaciones fisiopatológicas ha aumentado notablemente. En el compartimiento linfoide se ha descubierto un conjunto de células denominadas células linfoides innatas o *innate lymphoid cells* (ILC). Las ILC incluyen cinco grupos, clasificados según su similitud fenotípica y funcional con diferentes subpoblaciones de linfocitos T. A diferencia de los linfocitos T y B, las ILC no expresan receptores de antígeno ni sufren selección y expansión clonal cuando se activan. En cambio, responden rápidamente frente a citoquinas y señales de peligro en tejidos infectados o inflamados produciendo citoquinas que dirigen la respuesta inmune hacia un tipo adecuado para controlar la noxa original. Además, las ILC establecen un diálogo cruzado con otras células del microambiente que contribuye al mantenimiento y la restauración de la homeostasis tisular. Si bien muchas evidencias acerca de las ILC fueron obtenidas en modelos animales, existen datos sólidos que confirman su existencia en seres humanos y su papel en diversos trastornos inflamatorios. En este artículo, abordamos los nuevos conocimientos acerca de las ILC, y su rol en la homeostasis del sistema inmune y en diversas patologías inflamatorias, con el fin de presentar nuevos actores que regulan la inmunidad y la inmunopatología, lo que repercute en la salud humana.

Palabras clave: inmunidad innata, citoquinas, factores de transcripción, inflamación, homeostasis

While acute inflammation is a protective mechanism against infectious agents, chronic inflammation contributes to the progression of multiple pathological processes of infectious and non-infectious origin. Recently, a new family of innate immune cells called innate lymphoid cells (ILC) has been characterized, which plays an essential role in the initiation, regulation and resolution of inflammation. Unlike T and B lymphocytes, ILC lack antigen receptors and are rare in lymphoid tissues but enriched in mucous membranes and skin, where they also contribute to the maintenance and restoration of the integrity of epithelial barriers[1]. Most ILC do not express pattern recognition receptors and consequently, they are not directly activated in response to pathogen-associated molecular patterns. In contrast, they respond to cytokines, alarmins and inflammatory mediators derived from myeloid and epithelial cells, and produce immunoregulatory cytokines. ILC do not express markers of T and B lineage (they are $CD3^-$, $CD19^-$, $CD20^-$), myeloid cells, or granulocytes, and almost all express CD132 (common γ chain of the IL-2 receptor),

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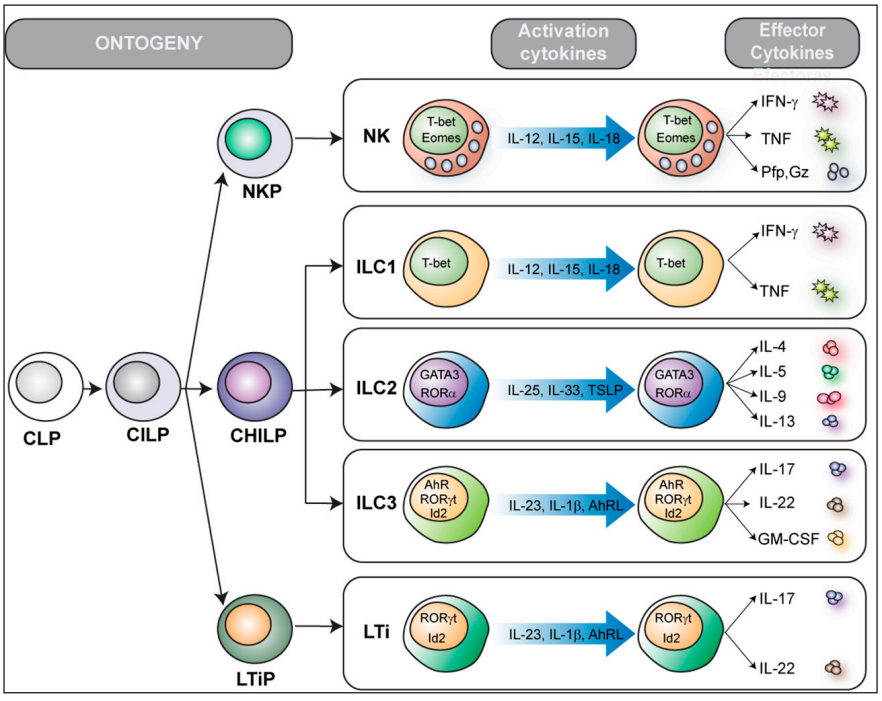
CD127 (IL-7Rα), CD25 (α chain of the IL-2 receptor), and CD90 (Thy1). The cytokines produced by ILC can also activate myeloid dendritic cells for which they exert effects on the adaptive immune response. Although these functions are beneficial to the host in a context of infection, in pathological processes, ILC can contribute to potentiate chronic inflammatory processes and tissue damage due to an exacerbated cytokine production. Recently, ILC have been classified into five subsets, following certain similarities with the classical profiles of cytokine expression and effector functions of T lymphocytes. These subsets are called natural killer (NK) cells, ILC1, ILC2, ILC3 and lymphoid tissue inducer cells or lymphoid tissue inducing (LTi) cells (Fig. 1).

NK cells and ILC1

Both types are activated in response to proinflammatory cytokines such as IL-12, IL-15 and IL-18, produce interferon (IFN)-γ and tumor necrosis factor (TNF) and express the activation receptors NKG2D and NKp46. Unlike NK cells, ILC1 lack most of the activation and inhibitory receptors of the Ly49 and KIR family[2], making the "missing self" recognition system exclusive of NK cells[3]. In addition, the cytotoxic machinery of NK cells (expression of granzymes and perforins) is absent in ILC1, so they are not cytotoxic. Therefore, NK cells could be considered the innate counterpart of CD8 T lymphocytes and ILC1, the innate counterpart of CD4 Th1 lymphocytes. In addition, while ILC1 are tissue-resident cells, NK cells circulate mostly in blood[4]. Therefore, ILC have properties similar to tissue-resident memory T cells (TRM)[5].

Both cell types express the transcription factor T-bet, which regulates many of their effector functions. The transcription factor Runx3 that is necessary to generate CD8 T cells is also necessary for the correct development and function of NK cells, ILC1 and ILC3 but not of ILC2[6]. However, only NK cells express the transcription factor eomesodermin[2].

Fig. 1.– Ontogeny, subsets, activation cytokines and effector cytokines that define innate lymphoid cells



AhR: arylhydrocarbon receptor; CHILP: common helper innate lymphoid cell progenitor; CILP: common innate lymphoid cell precursor; CLP: common lymphoid cell precursor; Eomes: eomesodermin; GATA3: binding protein 3 to DNA sequence [A/T]GATA[A/G]; GM-CSF: granulocyte-macrophage colony-stimulating factor; Gz: granzyme; Id2: inhibitor of DNA-binding protein 2; IFN-γ: interferon gamma; ILC: innate lymphoid cell; LTi: lymphoid tissue inducer cell; LTiP: lymphoid tissue inducer cell precursor; NKP: natural killer cell precursor; NK: natural killer cell; Pfp: perforin; RORα: retinoid-related orphan receptor α; RORγt: retinoid-related orphan receptor γt; T-bet: T-box transcription factor expressed in T cells; TNF: tumor necrosis factor; TSLP: thymic stromal lymphopoietin

ILC2

ILC2 are preferentially located in intestine, lung and skin epithelia, and are activated in response to cytokines such as IL-33, IL-25 and thymic stromal lymphopoietin (TSLP)[7]. ILC2 are non-cytotoxic cells that secrete cytokines characteristic of the Th2 profile (IL-4, IL-5, IL-6, IL-9, IL-13 and amphiregulin or Areg); they are characterized by the expression of the transcription factors GATA-3 and RORα, and molecules such as ICOS, CD25 and IL-1RL1 (the receptor for IL-33), and the surface receptor KLRG1[1]. Human ILC2 express some Toll-like receptors and respond to stimulation with specific ligands producing IL-5 and IL-13. They play an important role during the immune response to intestinal helminths, and in adipose tissue they regulate metabolic homeostasis and obesity, exerting regulatory effects on eosinophils and the development of anti-inflammatory macrophages that regulate insulin sensitivity[8]. ILC2 also regulate the differentiation of adipocyte progenitors towards white or brown fat, so they affect the body's thermal homeostasis[9].

ILC3 and LTi cells

LTi cells are localized in embryonic tissues, where they contribute to the organogenesis of lymph nodes and mucosa-associated lymphoid tissues. ILC3 comprise two subpopulations according to the expression of the natural cytotoxicity receptors (NCR) NKp46 or NKp44[10], named ILC3 NCR^+ and ILC3 NCR^-, and are located in mucosa-associated lymphoid tissues and in lamina propria. NCR^+ ILC3 cells produce IL-22, while NCR^- ILC3 cells produce IL-17, indicating that they are the innate counterparts of Th22 and Th17 cells. ILC3 and LTi express the transcription factor RORγt[1], which regulates the expression of IL-22, IL-17 and the arylhydrocarbon receptor, a transcription factor that is activated by recognition of ligands found in food, microflora or own cells. ILC3 NCR^+ cells seem to play an important role in the maintenance of the intestinal microbiota and the prevention of colonization by pathogenic bacteria, because IL-22 acts on cells of the intestinal epithelium, stimulating the turnover of enterocytes, and the production of antimicrobial peptides and mucus. IL-17-producing NCR^- ILC3s participate in immunity against *Candida albicans* in oral mucosa and may also exert pathogenic effects in colitis[1, 3]. In addition, IL-17 promotes the production of antimicrobial peptides and the recruitment of neutrophils. Therefore, the balance in ILC3 subpopulations is critical for the maintenance of mucosal homeostasis. It has also been shown that human ILC3 respond to stimulation with toll-like receptor agonists so that they may be activated directly by microbial stimuli.

Development of ILC

ILCs derive from a common lymphoid precursor and develop in the usual hematopoietic sites (fetal liver, bone marrow). This common lymphoid precursor differentiates towards a common precursor of ILC that gives rise to the NK cell precursor, the LTi cell precursor and the common helper ILC progenitor, which will eventually lead to the generation of ILC1, ILC2 and ILC3 (Fig. 1). These ILC precursors are $CD34^+$, $CD45RA^+$, $CD117^+$ and express the transcription factors TOX, Nfil3, TCF1 and Id2[1, 2, 7]. Id2 is critical to generate ILC because its sustained expression prevents differentiation into the T and B cell lineages. ILC1 differ from NK cells in that they express CD127 (IL-7Rα) but both express CD122 (IL-2/IL-15 receptor β chain), being IL-15 necessary to generate both lineages. The generation of ILC2 requires the transcription factors GATA-3, TCF1, RORα, ETS1 and Id2, the Notch receptor and the cytokines IL-25 and IL-33[10, 11]. Through the recognition of Notch ligands on bone marrow stromal cells, Notch promotes differentiation towards the ILC2 lineage. On the other hand, IL-25R and IL-33R confer the ability to sense and respond to the alarmins IL-25 and IL-33, promoting the secretion of Th2 cytokines (IL-4, IL-5, IL-13)[11]. In addition, retinoic acid derived from the metabolism of vitamin A is essential for the generation of LTi cells, ILC1 and ILC3 but not ILC2[12], while the expression of transcription factor RORγt is critical for the generation of ILC3 because it controls the expression of IL-17 and IL-22.

The generation of ILC occurs from embryonic life to adulthood and some evidence indicates that ILC residing in tissues derive from precursors that migrate early during embryogenesis. Either as multipotent precursors or committed to a single lineage, these cells conserve local self-renewal capacity throughout the life of the individual[4]. This situation would be similar to that of tissue macrophages, which develop from yolk sac-derived progenitor cells prior to definitive hematopoiesis and are able to self-renew locally throughout the life of the individual[13]. However, there is evidence indicating that ILC generated by hematopoiesis in the adult life can also repopulate various tissues in response to infectious or local inflammatory stimuli.

Plasticity and reciprocal regulation of ILC

As with CD4 T lymphocytes, plasticity has been demonstrated to occur in ILC. IL-12 and IL-18 can promote the conversion of ILC3 into IFN-γ-producing ILC1 and IL-17 can prevent this effect[11]. IL-1β and IL-23 can promote the conversion of ILC1 to ILC3, while IL-33 and IL-1β can promote the expression of T-bet in ILC2 and confer the ability to produce IFN-γ in response to IL-12. These reprogrammed ILC2 can retain the expression of GATA-3 and the production of IL-13 (ILC2/1) or lose GATA-3 expression

and become frank ILC1[11]. In addition, ILC1s can become NK cells if the expression of eomesodermin is induced[14].

There is also a reciprocal regulation between different ILC in a similar manner to what occurs between effector CD4 T lymphocyte subpopulations. ILC2 are inhibited by IFN-γ and by IL-27, cytokines that promote type 1 immunity, while IL-25 and TSLP, which promote the generation of ILC2, can suppress the secretion of IL-22 by ILC3.

ILC in homeostasis and pathology

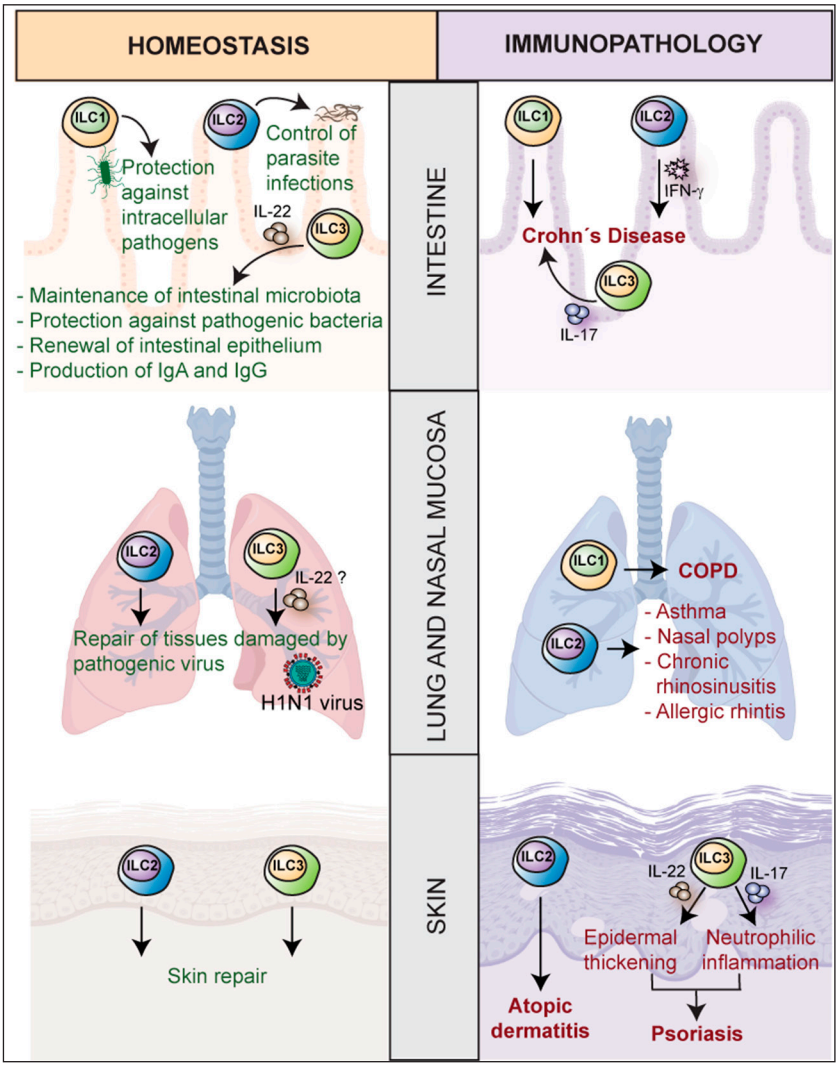
Intestine

ILC2 are detectable in the fetal intestine, but their frequency in the adult intestine is very low, while ILC3 are similarly frequent in the intestine of fetuses and adults. Likewise, ILC1 are barely detectable in the fetal intestine, and their development requires the stimulation by the microbiota[15]. The most prevalent ILC in the human intestine are IL-22-producing NCR$^+$ ILC3, which contribute to restrain commensal bacteria and the renewal of epithelial cells. ILC3 also favor IgA and IgG production in mucosa and spleen because they contribute to the activation of B lymphocytes through the production of cytokines such as BAFF and APRIL, and express CD40L[16] (Fig. 2).

In the human intestine, ILC1 are located either in the lamina propria (LP ILC1, NCR$^-$CD161$^+$CD127$^+$) or as intraepithelial ILC1 (ieILC1, NCR$^+$CD103$^+$CD127$^-$). In both locations they participate in the immunity against intracellular pathogens that enter through the mucosa[3].

The composition of the ILC populations changes markedly in inflamed human intestine (Fig. 2). IL-17-producing ILC3 are significantly enriched in inflamed ileum and colon

Fig. 2.– Functions of innate lymphoid cells in homeostasis and in different immunopathological contexts in intestine, lung and skin



of patients with Crohn's disease and mice with experimental colitis, and it is likely that this IL-17 contributes to the disease. In patients with Crohn's disease there is also an accumulation of ILC1 (LP ILC1 and ieILC1), and the magnitude of this increase in ileum reflects the severity of the disease[17]. The presence of IFN-γ-producing ILC2 has also been described, which is an evidence of ILC plasticity.

Lung

In human fetal lung tissue, NCR^-, IL-17-producing ILC3 predominate. ILC3 continue to be abundant in adult life but the frequency of ILC2 increases in comparison with fetal lung tissue (Fig. 2). Those ILC2 may mediate detrimental responses since the increased expression of genes such as IL-33 and its receptor, TSLP, IL-4, IL-5 and IL-13 (related to ILC2) are associated with susceptibility to atopic diseases such as asthma, atopic dermatitis and chronic rhinosinusitis. Skin lesions or nasal polyps of patients with atopic dermatitis or chronic rhinosinusitis show an enrichment in activated ILC2[18], while a greater number of ILC2 has been detected in peripheral blood and bronchoalveolar lavage fluid of patients with asthma[19], which correlates with a poorer respiratory function[20]. An increase in ILC2 has also been observed at different sites in patients with severe systemic eosinophilic asthma, in patients with allergic rhinitis sensitized against different allergens or in patients with allergic asthma[19]. It is believed that the cytokines TSLP, IL-4, IL-25 and IL-33 produced by epithelial cells and eosinophils stimulate ILC2 to produce IL-5 and IL-13, which activate eosinophils (abundant in nasal polyps), creating a positive feedback loop for chronic type 2 inflammation (Fig. 2).

IL-13 derived from ILC2 also affects lung function since it affects the contractility of smooth muscle cells of the respiratory tract, increases the production of mucus by epithelial cells, polarizes macrophages to an anti-inflammatory phenotype and increases collagen deposition. However, ILC2 not only play a pathogenic role, but also promote tissue repair in response to IL-33 produced upon influenza virus infection.

In patients with chronic obstructive pulmonary disease (COPD), reduced ILC2 numbers and increased ILC1 numbers have been described in lung tissues and blood, which would result from a reconversion of ILC2 into ILC1[18, 21] and this number correlates with poor pulmonary function, severe disease and susceptibility to acute exacerbations[21] (Fig. 2).

Skin

Human ILC are only found in the dermis. In humans, ILC2 produce mediators involved in skin repair, but ILC also appear to contribute to deregulated immune responses such as those observed in patients with atopic dermatitis and psoriasis. ILC2 are enriched in the skin with atopic dermatitis, pathology that is associated with increased expression of IL-25, IL-33, TSLP and PGD2, all of which promote the activation of ILC2. In addition, the epidermal thickening characteristic of psoriasis has been attributed to the production of IL-22, whereas neutrophilic inflammation is due to the production of IL-17, both cytokines produced locally by ILC3. Accordingly, an accumulation of ILC3 in skin and blood has been observed in patients with psoriasis[22] (Fig. 2).

Tumors

Although the role of NK cells in anti-tumor immunity is known, there are few data on the role of ILCs in the rejection of tumors. In humans, NCR^+ ILC3 are enriched in non-small cell lung carcinomas and have a favorable impact on the course of the disease[23]. In melanoma, it has been observed that NCR^+ ILC3 are stimulated by IL-12 and reconvert into IFN-γ producers, which promote rejection of the tumor, whereas in a colorectal cancer model IL-22 producing ILC3 were found to promote tumor formation, cell proliferation and growth induced by the chronic inflammation associated to colitis. In a breast tumor model, the generation of ILC1-like cells expressing granzyme B is important for the rejection of tumors. In gastric cancer, a predominantly Th2 phenotype correlates with a poor prognosis. In liver cancer, excessive production of IL-22 is associated with tumor growth and metastasis, suggesting a role for ILC3 in this type of cancer. In summary, there is preliminary evidence on ILC playing a dual role in anti-tumor immunity[24]. However, the mechanisms by which ILCs interact with malignant cells remain unknown.

Metabolic regulation

Detrimental effects mediated by ILC2 have been described in tissue remodeling in the context of hepatic fibrosis, since IL-13 produced in response to IL-33 induces fibrosis mediated by hepatic stellate cells[25]. On the other hand, the white adipose tissue shows enrichment in ILC2 that seem to prevent the development of brown fat through the production of IL-5 and IL-13 that operate on the generation of adipocytes, promote the recruitment and local activation of eosinophils and the generation of anti-inflammatory macrophages[1]. Therefore, ILC also participate in the regulation of thermogenesis in adipose tissues but their importance in the human metabolic syndrome is not yet fully understood.

Conclusions

The discovery of ILC has revealed an ancestral innate immune system that gave rise to adaptive immunity. The

effector cells generated, whether they are ILC or T lymphocytes, have preserved the effector mechanisms (cytokine production and cytotoxic responses) due to the expression of a similar pattern of transcription factors, which allows them to eliminate pathogens and restore homeostasis. The advantage of ILC is that they quickly translate the signals produced by infected or injured tissues into cytokines that activate and regulate local innate and adaptive effector functions. Like lymphocytes, ILC also contribute with inflammatory components to various pathological conditions. On the other hand, as tissue-resident cells, ILC exert regulatory effects at different anatomical sites. Therefore, ILC emerge as potential therapeutic targets to manipulate their activity in favor of human health.

Conflicts of interests: None to declare

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