研究 CD43 在 CD8+ T 淋巴球活化過程中之作用

Study of the function of CD43 in CD8+ T lymphocytes activation

中文摘要

CD43 是一種穿膜醣蛋白，大量表現在 T 淋巴球的表面上。先前的研究認為 CD43 參與 T 細胞的活化，但其為促進或抑制活化的角色，仍具爭議。在本實驗室先前的研究中，利用固定抗體在細胞培養盤上的刺激方式刺激 T 細胞，發現在給予抗 T 細胞受體及 IL-2 的情況下，抗 CD43 抗體的刺激會進一步增加 CD8+ T 淋巴球的增殖，卻不影響 CD4+ T 淋巴球的增殖，建議 CD43 在 CD8+ T 淋巴球的活化過程中有輔助功能。此外，在脾臟 CD8+ T 淋巴球上 CD43 的表現量高於 CD4+ T 淋巴球，CD43 的特殊表現模式及其對 CD8+ T 淋巴球增殖的可能影響，促使我進一步研究 CD43 在 CD8+ T 淋巴球活化中的角色。我的實驗設計是以非抗 CD43 抗體刺激的方式來研究 CD43 在 CD8+ T 淋巴球活化過程中的作用。首先，我利用非抗原專一性的體外刺激方式，以除去 T 細胞的正常脾臟細胞外加不同濃度抗 CD3 抗體來刺激 CD8+ T 淋巴球，分別比較野生型小鼠及 CD43 基因剔除(CD43-/-)小鼠 CD8+ T 淋巴球的增生程度，發現在低濃度抗 CD3 抗體刺激下，野生型 CD8+ T 淋巴球的增生程度高於 CD43-/-CD8+ T 淋巴球。然而在試混合淋巴球反應(mixed lymphocytes reaction, MLR)中，CD43 的缺損並不會降低 CD8+ T 淋巴球的活化以及增殖。此外，利用專一性抗原(antigen-specific)冇菖鏈及骨髓衍生性樹突細胞(BM-Dc)刺激 CD8+ T 淋巴球，我們發現在不成熟 BM-Dc 刺激下，可以偵測到 CD43 輔助的作用，但是在成熟 BM-Dc 刺激下則否。我進一步利用外加 CTLA-4-IgFc 的方式來證明 mature BM-Dc 與 immature BM-Dc 作用的差異，並非源自 mature BM-Dc 上大量 B7 分子的作用遮蔽住 CD43 促進 CD8+ T 淋巴球增殖的功能，而且在本文測試系統下，CD43 的作用無法獨立於 CD28 與 B7 所造成的 costimulation 之外。

先前的研究指出，在 CD4+ T 淋巴球與抗原呈現細胞相互作用時，CD43 會被排除在兩個細胞所共同形成的免疫突觸(Immunological Synapse, IS)結構之外，但在 CD8+ T 淋巴球上則是未知。我在 OT-1 TCR transgenic 系統下以專一性抗原刺激 CD8+ T 淋巴球活化，觀察 CD43 的分佈，卻發現有超過半數的活化 CD8+ T 淋巴球，其 CD43 與 TCRb 共同座落在兩細胞所形成的交界處，這也意味著 CD43 可能參與 CD8+ T 淋巴球的活化。

英文摘要

CD43 is a transmembrane sialogycoprotein expressed on the surface of a variety of hematopoietic cell, including T lymphocyte. Previous studies suggested that CD43 might be involved in T lymphocyte activation. Both positive and negative effects of CD43 are reported, but its definite function remains controversial. Our earlier study

showed that costimulation of naïve CD8+ T lymphocytes with plate-bound a-CD43 monoclonal antibody significantly enhances the proliferation response to TCR stimulation in the presence of exogenous IL-2. This result suggests that CD43 might help the activation of CD8+ T lymphocytes. Others and we also found that the expression of CD43 in splenic CD8+ T lymphocytes is uniformly higher than that on CD4+ T lymphocytes. Three results prompted me to further investigate the role of CD43 in the activation of CD8+ T lymphocytes without using a-CD43 antibody stimulation. Three stimulation systems were used in my study. Firstly, CD8+ T lymphocytes were stimulated with T lymphocyte-depleted splenocytes plus various amounts of anti-CD3 antibody. We found that the proliferation of wild type CD8+ T lymphocytes was higher than that of CD43-/- CD8+ T lymphocytes at low concentrations of anti-CD3 antibody. Secondly, CD8+ T lymphocytes were stimulated with allogeneic spleen cells. We found that the deficiency of CD43 did not attenuate the proliferation of CD8+ T lymphocytes. Thirdly, by using OT-1 TCR transgenic system, the CD8+ T lymphocytes were stimulation by antigen-specific peptide-pulsed dendritic cells. We found that the proliferation of wild type CD8+ T lymphocytes was higher than that of CD43-/- CD8+ T lymphocytes.
Since CD43 is a large and highly negatively charged protein that extends in a linear conformation outward from the cell membrane, it was suggested that CD43 might function as a barrier for T lymphocyte-APC interaction. Several studies demonstrated that CD43 was excluded from the immunological synapse between CD4+ T lymphocyte and APC. We observed that the exclusion of CD43 from the T/APC contact site during CD8+ T lymphocyte activation is not obligatory in OT-1 TCR transgenic system. These results suggest that CD43 may play a positive role in regulation of CD8+ T lymphocyte activation.