Available online at www.sciencedirect.com
ScienceDirect
Comparative Immunology, Microbiology and Infectious Diseases 32 (2009) 419–428
COMPARATIVE IMMUNOLOGY MICROBIOLOGY & INFECTIOUS DISEASES
www.elsevier.com/locate/cimid

Characteristic cellular composition of germinal centers

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Accepted 29 December 2007

Abstract

We established the method of isolating individually encapsulated germinal centers (GCs) from immunized spleen and analyzed single cell suspension of GCs by flowcytometry. In GCs, the high frequency of $sIgG^+$ cells (29%) and $sIgA^+$ cells (5%) was detected. Two-color flowcytometry analysis showed that GCs contained 27% of $sIgM^-IgG^+$ cells, in which isotype switch from IgM to IgG had occurred, and 5% of $Bu1^-IgG^+$ cells, which were differentiating into plasma cells. On the other hand, $sIgM^-IgG^+$ and $Bu1^-IgG^+$ cells were not detected in the bursa, which contained 95% of B cells and only 1% of T cells. $CD4^+$ but not $CD8^+$ T cells were detected in the light zone of GCs and these $CD4^+$ T cells are supposed to play a key role in isotype switching and differentiation into plasma cells in GCs. These results clearly demonstrate that GCs provide a site for isotype switching and differentiation into plasma cells.

Keywords: Germinal center; Chicken; Isotype switching; Plasma cell; T cell

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0147-9571/$ – see front matter
doi:10.1016/j.cimid.2007.12.002

Résumé

Ayant établi la méthode d'isolement de centres germinaux encapsulés (GCs) individuels issus de la rate, nous avons analysé les suspensions cellulaires de ces GCs par cytométrie de flux. Dans les GCs, une fréquence élevée de cellules $sIgG^+$ (29%) et $sIgA^+$ cells (5%) était détectée. L'analyse en cytométrie de flux à deux couleurs a montré que les GCs contenaient 27% de cellules $sIgM^-IgG^+$, dans lesquelles le passage de l'isotype IgM vers l'isotype IgG avaient eu lieu, et 5% de cellules $Bu1^-IgG^+$ qui se différenciaient en plasmocytes. À l'inverse des cellules T $CD8^+$, les cellules T $CD4^+$ étaient détectées dans la zone claire des GCs. Ces cellules T $CD4^+$ sont supposées jouer un rôle clef dans le changement d'isotype et la différenciation des plasmocytes dans les GCs. En contraste, les populations cellulaires $sIgM^-IgG^+$ et $Bu1^-IgG^+$ n'étaient pas détectées dans la bourse qui contient 95% de cellules B et seulement 1% de cellules T. Ces résultats établissent clairement que les GCs constituent un site clef pour le changement d'isotype des immunoglobulines et la différenciation des plasmocytes.

Mots clés : centre germinal ; poulet ; changement d'isotype ; plasmocyte ; cellule T

1. Introduction

Germinal centers (GCs) are histologically defined lymphoid follicles in peripheral lymphoid organs after antigenic stimulation [1–3]. They are found both in mammals and birds (homoiothermic animals) [1,4,5]. GCs are composed of a large number of B cells, a few T cells and follicular dendritic cells (FDCs) [1–3,5,6]. The microenvironments in GCs induce gene modifications such as somatic hypermutation, gene conversion or secondary immunoglobulin (Ig) gene rearrangement in GC B cells [7–9]. Mutated B cells are thought to be selected by antigens retained on FDCs and undergo isotype switching or differentiations into memory B cells or plasma cells [1,10]. Immune complexes on FDCs are predominantly formed with IgG or IgA [11]. T cells in GCs are thought to be necessary for further differentiation of GC B cells. Especially, T cells or cytokines are supposed to be prerequisite to the escape from apoptosis and isotype switching in GCs [1,2,12].

GCs contain two histologically identifiable zones: the dark zone (DZ) and the light zone (LZ) [1,2,5,12]. The DZ is constituted of proliferating B cells, which are surface Ig (sIg) negative [12]. The LZ is composed of a few helper T cells, non-dividing B cells and FDCs. In the LZ, B cells are supposed to undergo isotype switching or differentiate into plasma blasts.

In chickens, GCs are found as B-cell follicles at the bifurcation of arterioles in spleen of immunized chickens (Fig. 1A) [13]. Chicken splenic GCs are circumscribed by a capsule of connective tissue and associate with arteriores [6,13]. Therefore, it is possible that chicken GCs are separated from chicken spleen [6,7,13,14].

In tissue sections, the isotype of sIg expressed on B cells is difficult to analyze due to the presence of immune complexes, which are formed with IgG or IgA [1]. In the present study, we established the methods of isolation of single GCs from spleen. We examined phenotypes of GC cells derived from isolated single GCs, and especially analyzed surface Igs expressed on GC B cells using double immunofluorescence.