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Research paper

Isolation of functionally active murine follicular dendritic cells☆

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Abstract

Biochemical, genetic, and immunological studies of follicular dendritic cells (FDCs) have been hampered by difficulty in obtaining adequate numbers of purified cells in a functional state. To address this obstacle, we enriched FDCs by irradiating mice to destroy most lymphocytes, excised the lymph nodes, and gently digested the nodes with an enzyme cocktail to form single cell suspensions. The FDCs in suspension were selected using the specific mAb FDC-M1 with magnetic cell separation technology. We were able to get nearly a million viable lymph node FDCs per mouse at about 90% purity. When examined under light and transmission electron microscopy, the cytological features were characteristic of FDCs. Furthermore, the cells were able to trap and retain immune complexes and were positive for important phenotypic markers including FDC-M1, CD21/35, CD32, CD40, and CD54. Moreover, the purified FDCs exhibited classical FDC accessory activities including: the ability to co-stimulate B cell proliferation, augment antibody responses induced by mitogens or antigens, maintain B cell viability for weeks, and protect B lymphocytes from anti-FAS induced apoptosis. In short, this combination of methods made it possible to obtain a substantial number of highly enriched functional murine FDCs.

Keywords: Follicular dendritic cells; Magnetic bead purification; FDC-M1; FDC phenotype; Isolation procedure; Electron microscopy

1. Introduction

Follicular dendritic cells (FDCs) are important immune accessory cells that reside in the follicles of secondary lymphoid organs and are functionally active in the light zones of germinal centers (GCs) where they are associated with proliferating B cells. Their unique ability to trap and retain surface bound immune complexes (ICs) together with their restricted follicular location, distinguishes FDCs from all other accessory cells including T cell associated dendritic cells (DCs) (Tew et al., 1982). FDCs bearing specific Ag in ICs are requisite for full development of GCs and are believed to be involved in T-dependent B cell responses: Ig class switching, production of B memory cells, selection of somatically mutated B cells with high affinity antigen receptors (BCR), affinity maturation and augmentation of secondary Ab responses (Tew et al., 2001). In

Abbreviations: FDC, follicular dendritic cells; mAb, monoclonal antibody; ICs, Immune complexes; PO, peroxidase; OVA, ovalbumin; HRP, horseradish peroxidase.

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addition, ICs trapped on FDCs include large amounts of infectious HIV particles which can persist for prolonged periods of time (Racz et al., 1989; Smith et al., 2001). Retention of HIV particles on FDCs plays an important role in HIV pathogenesis and constitutes a potent reservoir present adjacent to $CD4^{+ve}$ T cells (Burton et al., 1997, 2002; Piris et al., 1987; Fox et al., 1991; Racz et al., 1989; Heath et al., 1995). FDCs have also been implicated in the pathogenesis of transmissible spongiform encephalopathies (TSE) caused by prion proteins. Strong evidence for the accumulation of pathological prion protein on FDCs exists (Fraser and Farquhar, 1987; Kitamoto et al., 1991; McBride et al., 1992). Furthermore, the presence of functional FDCs appears to be important for lodgment, replication and further spread of the infective prion protein (Brown et al., 1999; Mabbott et al., 2000; Manuelidis et al., 2000). Thus, we reason that an appreciation of FDC accessory activities and the regulation of these activities are critical to an understanding of fully functional and mature Ab responses as well as diseases associated with FDCs.

Compared with the other immune cells, little genetic and biochemical information is available on FDCs. This lack of information is attributable in large measure to FDCs being: 1 — rare (~1 in 10,000 cells in secondary lymphoid tissues), 2 — very fragile, and 3 — challenging to obtain in a functional state in adequate number. Consequently, very few laboratories work with FDCs. Our knowledge of more typical leukocytes has been largely derived from *in vitro* studies using purified populations. We reason that efforts to characterize biochemical and genetic properties of FDCs would expand if reasonably simple methods were developed to isolate adequate numbers of functional FDCs in purity. FDCs from humans and mice have been enriched in various states of purity and studied (Lilet-Leclercq et al., 1984; Tsunoda et al., 1990; Wu et al., 1996; Marcoty et al., 1993; Heinen et al., 1993; Stahmer et al., 1991; Parmentier et al., 1991; Sellheyer et al., 1989; Ennas et al., 1989; Cormann et al., 1988; Heinen et al., 1985; Schmitz et al., 1993; Humphrey and Grennan, 1982; Clark et al., 1992). Furthermore, FDC-M1 has been used to positively select for murine FDCs using two cycles of cell sorting (Burton et al., 1993) as well as to deplete murine FDCs from enriched preparations (Burton et al., 1993; Wu et al., 1996; Huber et al., 2005). However, methods routinely used to obtain functional murine FDCs are based on density gradients and yield preparations in the range of 25% to 50% purity (Wu et al., 1996; Burton et al., 1993). Nevertheless, these enriched preparations have been useful in studies which showed that FDCs: 1) cluster with B cells and promote proliferation of adjacent B cells (Kosco et al., 1992); 2) block apoptosis in B cells (Schwarz et al., 1999) and maintain viable B cells and a functioning immune system *in vitro* for weeks (Qin et al., 1999); 3) block the ITIM signaling in B cells stimulated by ICs (Aydar et al., 2004, 2003); 4) provide co-stimulatory signals for B cell proliferation stimulated by antigen or mitogen (Burton et al., 1993; Tew et al., 2001; Qin et al., 1998), and 5) promote recall IgG responses (Wu et al., 1996; Fakher et al., 2001). However, 25 to 50% enrichment is not adequate for biochemical, genetic, or immunological studies aimed at determining the molecules critical for accessory functions.

Accordingly, we sought to improve purity and yield while maintaining immunological function. Magnetic cell separation, a relatively simple and inexpensive method, has been successfully used to isolate a variety of rare cells including human FDCs (Schmitz et al., 1993). We first used established methods to enrich murine FDCs, which included killing radiosensitive lymph node cells by irradiation (Phipps et al., 1981; Kosco-Vilbois et al., 1993) and an enzyme cocktail to obtain single cell suspension (Schnizlein et al., 1985). Finally, we used FDC-M1, a monoclonal antibody specific for murine FDCs (Kosco et al., 1992; Gray et al., 1991), to label FDCs, which were then positively selected in a magnetic column. Using this combination of techniques with the harvested lymph nodes, we obtained about a million viable lymph node FDCs per mouse. Flow cytometric analysis indicated that about 90% of these cells exhibited the FDC phenotype and that very few contaminating macrophages and lymphocytes were present. We also found that positively selected FDCs maintained their ability to bind and retain surface ICs, protect B cells from Fas/CD95 mediated apoptosis, enhance proliferation of mitogen stimulated B cells, and augment antibody production in mitogen or antigen stimulated B cells. In addition, this methodology was used to positively select FDCs in a published study to determine the influence of IC bearing FDCs on the IgM response, Ig class switching, and production of high affinity IgG (Aydar et al., 2005). In conclusion, the protocol described here enables the isolation of functional FDCs that are suitable for a variety of studies including biochemical and genetic analysis (Sukumar et al., 2006).

2. Materials and methods

2.1. Mice, immunization, and irradiation

BALB/c mice, 6 to 8 weeks old, were purchased from the National Cancer Institute. They were housed