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Phenotypic characterisation of immune cell infiltrates in testicular germ cell neoplasia

Tine Hvarness[a], John E. Nielsen[a], Kristian Almstrup[a], Niels E. Skakkebaek[a], Ewa Rajpert-De Meyts[a,*], Mogens H. Claesson[b,**]

[a] Department of Growth and Reproduction, Copenhagen University Hospital, Section 5064, Blegdamsvej 9, DK-2100 Copenhagen, Denmark
[b] Department of International Health, Immunology and Microbiology, Faculty of Health and Medical Sciences, University of Copenhagen, Blegdamsvej 3, DK-2200 Copenhagen, Denmark

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ABSTRACT

Immune cells often infiltrate testicular germ cell neoplasms, including pre-invasive carcinoma in situ (CIS), but the significance of this phenomenon remains unknown. The composition and distribution of infiltrating immune cells were examined by immunohistochemistry in testis samples with CIS and overt seminoma, in comparison to biopsies from infertile men without neoplasia. The composition of immune cells was similar across all the groups studied. Macrophages, $CD8^+$ and $CD45R0^+$ T lymphocytes constituted the majority of infiltrates, B lymphocytes were present in an intermediate proportion and very few $CD4^+$ and $FoxP3^+$ T cells were detected. HLA-I antigen was more abundant in Sertoli cells in tubules containing CIS than in those with normal spermatogenesis. This study showed a phenotypically comparable composition of infiltrating immune cells independently of the presence of neoplasia, suggesting the absence of active immune surveillance in testicular germ cell cancer.

1. Introduction

Testicular cancer is the most common cancer among young men aged 20–34 years, and more than 95% of testicular tumours are germ cell tumours (TGCT). TGCT of adolescents and young adults consist of seminomas and non-seminomas. Seminomas are histologically homogeneous, while non-seminomas are a heterogeneous group of tumours that may contain one or several components including embryonic carcinoma (the most undifferentiated type), yolk sac tumour, choriocarcinoma or teratoma (Oosterhuis and Looijenga, 2005).

Testicular germ cell tumours are believed to originate from the pre-invasive intratubular cell, carcinoma in situ (CIS) (Skakkebaek, 1972), also known as intratubular germ cell neoplasia unclassified, ITGCNU. CIS cells closely resemble foetal gonocytes, displaying many embryonic stem cell features, e.g. in the morphology and expression of immunohistochemical (IHC) markers, including expression of pluripotency factors (Jørgensen et al., 1995; Rajpert-De Meyts et al., 2003; Almstrup et al., 2004; Sonne et al., 2009). The risk of CIS is increased in individuals with signs of testicular dysgenesis syndrome (TDS) including some forms of male infertility (Skakkebaek et al., 2001).

Since the 1970s it has been observed that cells of the immune system infiltrate testis tissue with TGCT. These infiltrates are more often observed in seminomas, as opposed to non-seminomas, and are believed to correlate with the favourable prognosis of this cancer type (Ioachim, 1976; Mostofi and Sesterhenn, 1978; Parker et al., 2002). The infiltrates in seminomas and CIS adjacent

* Corresponding author. Tel.: +45 3545 8145; fax: +45 3545 6054.
** Corresponding author. Tel.: +45 3532 7272; fax: +45 3532 7269.
E-mail addresses: erm@rh.dk (E. Rajpert-De Meyts), claesson@sund.ku.dk (M.H. Claesson).

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to seminomas have been shown to consist mainly of T lymphocytes and macrophages while NK cells and B lymphocytes are present in lower numbers, the latter forming foci resembling lymphoid follicles (Bell et al., 1987; Nakanoma et al., 1992; Bols et al., 2000). A similar composition of the infiltrate has been reported in mixed non-seminomas (Gemmell et al., 1988).

Attempts have been made to elucidate which putative signal transduction pathways could be involved in the interaction between the tumour and the infiltrating immune cells in the testis. These include FasL expression (Kersemaekers et al., 2002; Baldini et al., 2009) and the absence/downregulation of HLA class I/II molecules by CIS/tumour cells, the latter, however, with conflicting results (Bell et al., 1987; Klein et al., 1990; Nouri et al., 1993; Tomita et al., 1993). Sertoli cells in tubules with both CIS and intratubular seminoma have been shown to express HLA class I molecules and might be doing so as a response to inflammatory cytokines released by infiltrating immune cells, because Sertoli cells in normal testes do not express HLA class I molecules (Bell et al., 1987; Braendstrup et al., 1995; Braendstrup, 1996).

Cytotoxic ($CD8^+$) T cells comprise a major component of infiltrating lymphocytes in solid tumours, particularly seminomas. However, the biological significance of this observation remains a subject of considerable debate as this cell type has been found to achieve a higher 'activation status' in seminomas than in non-seminomas (Yakirevich et al., 2002), but has also been suggested to be a 'low-activity' lymphocyte in CIS and seminoma (Bols et al., 2000). It is relevant to mention that in addition to cytotoxicity, CD8+ T cells may include cells with regulatory effector functions (Kim et al., 2010). The testes are regarded as an immunologically privileged site in which the blood–testis barrier and several immunosuppressive mechanisms ensure the survival of haploid germ cells expressing potential auto-antigens (Head et al., 1983; Itoh et al., 1999; Page et al., 2006; Fijak et al., 2011). It has been reported previously that levels of transcripts associated with inflammation (such as cytokines) correlated positively with the severity of spermatogenic failure – perhaps reflecting a gradual breakdown of testicular immune privilege (Spiess et al., 2007). T lymphocytes are probably involved in the disruption of immune privilege and several T cell subsets have been defined as regulators of this process, including T_{regs}, $CD8^+$ T cells and Th17, a pro-inflammatory subset of $CD4^+$ cells. However, the specific immunopathogenic mechanisms responsible for T cell infiltration are not known (Jacobo et al., 2009; Duan et al., 2011; Fijak et al., 2011).

Chronic inflammation can result from low grade, persistent chemical, bacterial and viral agents (Raman et al., 2007). The plethora of cell types, chemo- and cytokines in chronic inflammation has been shown to contribute to tumourigenesis at all stages, hence the term oncogenic inflammation (Grivennikov et al., 2010). It has been proposed that oncogenic inflammation and anti-tumour immunity (immune surveillance) are mutually exclusive processes (Balkwill and Mantovani, 2001), however, the co-existence of these two processes has been proposed in rodent studies (Teng et al., 2010; Zaidi et al., 2011). Thus, anti-tumour immunity and inflammation may inhibit or promote cancer development respectively, as tumour cells develop and transit through cancer immuno-editing (Bui and Schreiber, 2007; Schreiber et al., 2011).

In the present study we attempt to shed some light on the main outstanding question – whether the infiltrating cells in the early pre-invasive stages of TGCT are a part of an inflammatory/autoimmune response as a result of a breakdown of immune privilege, or whether they reflect a specific anti-tumour response associated with immune surveillance. To address this question, we first characterised the cell type/subtype of the infiltrating cells in testes with overt classical seminoma, as this cancer type is associated with a prominent immune cell infiltrate (Ioachim, 1976). Subsequently, we investigated cellular phenotypes, infiltration and HLA class I expression in specimens with CIS in different histological patterns, encompassing CIS alone and CIS adjacent to different TGCTs. Because of a known association between CIS and poor testis function and infertility, we also investigated testis biopsies from infertile men without CIS or TGCT. Finally, we assessed whether the immune cell composition and distribution changed depending on the presence and histological type of germ cell neoplasia.

2. Materials and methods

2.1. Patient samples

All tissue samples (frozen and paraffin-embedded) originated from patients with testicular cancer (orchiectomy specimens) and men undergoing testicular biopsies during andrological work-up of infertility. After routine pathological assessment, the tissues were deposited in the biobank at the Department of Growth and Reproduction, Copenhagen University Hospital. All patients had given consent to the use of archived tissues for research projects, which was approved by the Regional Medical Ethics Committee. Paraffin-embedded tissue samples for IHC (48 in total) included: seminoma ($n = 12$), CIS without tumour ($n = 4$), CIS adjacent to seminoma ($n = 9$), CIS adjacent to non-seminoma ($n = 8$), CIS adjacent to combined germ cell tumour (CGCT) ($n = 2$), testis biopsies from infertile men where the presence of inflammatory cell infiltrates was noted ($n = 11$) and control specimens from men from couples with idiopathic infertility and suspected obstructive forms (displaying normal testis histology and no inflammatory changes), NT ($n = 2$).

Tissue samples had been fixed either in formalin, GR fixative (modified Stieve's) or Bouin's fixative. According to the initial histopathological evaluation, all paraffin-embedded biopsies expressed signs of infiltration, except control specimens. In two cases, tissue blocks from the same patient were included in two different groups: one as a seminoma, the other as CIS adjacent to a seminoma (indicated by an asterisk in Fig. 3). Clinical variables such as age and histopathological findings for all patients are provided in Table 4. Additional information regarding the type of neoplasia is provided in Supplementary Table 1.