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An immunological study of germinal centres in four ophthalmic immunocytomas

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Summary. Four ophthalmic lymphoplasmacytic/lymphoplasmacytoid (LP) immunocytomas with germinal centres were reviewed histologically and studied immunologically by means of the peroxidase-antiperoxidase (PAP) method. In two cases a histological diagnosis of LP immunocytoma was made, while in the other two cases a non-Hodgkin's lymphoma could not be histologically differentiated with certainty from a pseudolymphoma or reactive process. Immunological analysis confirmed the diagnosis in the former two cases and led in one of the latter also to a diagnosis of LP immunocytoma. In the fourth case the development of LP immunocytoma out of a pseudolymphoma could be demonstrated. In the four LP immunocytomas the germinal centres showed a monoclonal pattern of immunoglobulin in one case, a polyclonal pattern in one case and a negative reaction in two cases. The role of germinal centres in relation to the development of LP immunocytoma is discussed.

Key words: Ophthalmic LP immunocytoma – Pseudolymphoma – Germinal centres – Intracytoplasmic immunoglobulin

Introduction

In the eye region the differential diagnosis between low-grade malignant non-Hodgkin's lymphoma, pseudolymphoma and reactive processes is generally known to be difficult. Well-defined borders, polymorphism of the infiltrate and the presence of germinal centres are said to favour a benign diagnosis (Meyer et al. 1970; Garner 1973; Schwarze et al. 1976; Astarita et al. 1980). We observed, however, that some ophthalmic lymphomas with the cytohistological characteristics of non-Hodgkin's lymphoma, especially lymphoplasmacytic/lymphoplasmacytoid (LP) immunocytoma, showed germinal centres. Therefore, all ophthalmic immunocytomas and ophthalmic

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lymphomas with a doubtful histological diagnosis seen at the Lymph Node Registry in Kiel between 1970 and 1979 were screened for the presence of germinal centres. Such cases were studied immunologically with the peroxidase-antiperoxidase (PAP) method in order to determine whether the plasma cells in the diffuse lymphoproliferative areas contain monoclonal immunoglobulin (Ig), to identify the Ig types in the germinal centres and to investigate a possible relation between the Ig type in the germinal centres and that of the plasma cells in the suspected lymphoma.

Material and methods

Among 30 ophthalmic immunocytomas, 6 showed germinal centres. Three were discarded because paraffin blocks were no longer available or insufficient. In a fourth case germinal centres were absent in the sections destined for PAP staining. The remaining 2 cases were studied (cases 1 and 2), including 4 follow-up biopsies in one of them (Table 1). In one case a benign process could not be distinguished with certainty from LP immunocytoma (case 3) and in another case (case 4) a pseudolymphoma was diagnosed, but in follow-up biopsies the question arose as to the benign nature of the tumour.

The 4 cases were reviewed cytohistologically in paraffin sections stained with haematoxylin and eosin, Giemsa, periodic acid Schiff (PAS) and silver impregnation (Gomori). For immunological examination the PAP method of Sternberger et al. (1970) in the modification of Mepham et al. (1979) was applied to paraffin sections as described in more detail elsewhere (Schmid et al. 1982). All cases were studied for the presence of intracytoplasmic Ig (κ, λ, IgG, A, M) and albumin.

Results

Patients. Three of the patients were female, one was male. All were over 60 years of age (Table 1). At the time of diagnosis no other tumour localizations were known.

Cases with a diagnosis of LP immunocytoma (Table 1). In *case 1* the tumour in the conjunctiva of the upper eyelid consisted of several well-circumscribed tumour lobules with additional, partly perivascular, patchy infiltrates. Cytologically, the tumour was composed of lymphocytes, plasmacytoid cells and plasma cells with scattered mast cells. PAS-positive inclusions were observed frequently, both in the cytoplasm and in the nuclei. In addition, focal accumulations of centroblasts and centrocytes were found; these were relatively well circumscribed and regarded as germinal centres. A histological diagnosis of LP immunocytoma was made. Immunoperoxidase staining revealed a monoclonal pattern of IgM/λ throughout the tumour and in the germinal centres (Fig. 1).

In *case 2* five biopsies were taken from a subconjunctival tumour within a period of $2^1/_2$ years. In none of them could the borders of the tumour be judged. In all biopsies, the proliferation consisted of lymphocytes, plasmacytoid cells and a varying number of plasma cells and mast cells. In the first biopsy many well-defined germinal centres were present. In the subsequent two biopsies, however, these were less pronounced and in the most recent two biopsies they were entirely absent. In all specimens intracy-

Table 1. Summary of patients' data and the main histological and immunological findings in four cases of ophthalmic lymphoplasmacytic/lymphoplasmacytoid (LP) immunocytoma

Case No.	Age	Sex	Interval between biopsies[a]	Initial diagnosis	Germinal centres	Immunostaining restuls		LP-IC[b]
						Diffuse areas	Germinal centres	
1	75	F		LP immunocytoma	Well developed	IgM/λ	IgM/λ	+
2	76	F		LP immunocytoma	Well developed	ND[c]	ND	+
			7 mos	ditto	Less pronounced	ND	ND	+
			10 mos	ditto	Less pronounced	IgM, A/κ	Negative	+
			15 mos	ditto	Absent	IgM/κ		+
			18 mos	ditto	Absent	IgM/κ		+
3	64	M		LP immunocytoma? Pseudolymphoma?	Remnants	IgM/κ	Negative	+
4	69	F		Pseudolymphoma	Pronounced	IgG, M/κ, λ	IgG, M/κ, λ	−
			5 years	ditto	ditto	IgG, M/κ, λ	IgG, M/κ, λ	−
			5 years	ditto	ditto	IgG, (M)/λ, (κ)	IgG, M/κ, λ	+/−
			8 years	Pseudolymphoma? LP immunocytoma?	ditto	IgG/λ	IgG, M/κ, λ	+

[a] Interval between first biopsy and follow-up biopsy
[b] LP-IC = final diagnosis of LP immunocytoma
[c] ND = not done

toplasmic and especially intranuclear PAS-positive inclusions were found. Histologically, a diagnosis of LP immunocytoma was made on all biopsies.

Immunoperoxidase staining could be performed on only the most recent three biopsies. In all of them only κ light chains were present in the plasmacytoid cells of the tumour, while both μ and α heavy chains were present in the first biopsy and exclusively μ in the other two. The germinal centres in the first of the three studied biopsies were negative for all Ig types.

Cases with a doubtful histological diagnosis (Table 1). In *case 3* all available material consisted of tumour tissue and the borders could therefore not be judged. Within the lymphoid proliferation remnants of lacrimal gland ductules were seen. Cytologically, the tumour was polymorphic, i.e. somewhat nodular areas of small lymphocytes and plasma cells were accompanied by strands of medium-sized lymphoid cells with irregular nuclei and large, intensely basophilic blast cells. Intracytoplasmic PAS-positive inclusions were found frequently and intranuclear inclusions occasionally (Fig. 2). In addition, foci of centroblasts and centrocytes were found; these resembled remnants of germinal centres. Histologically, a clear-cut diagnosis could not be made, and in view of the polymorphism a benign process was considered.

Immunoperoxidase staining revealed strong monoclonal intracytoplasmic positivity for IgM/κ in the lymphocytic-plasmacytic areas, while the areas with medium-sized lymphoid cells and blast cells and the germinal centre remnants were negative. In view of the monoclonality of the plasma cells, a final diagnosis of LP immunocytoma was made in case 3.

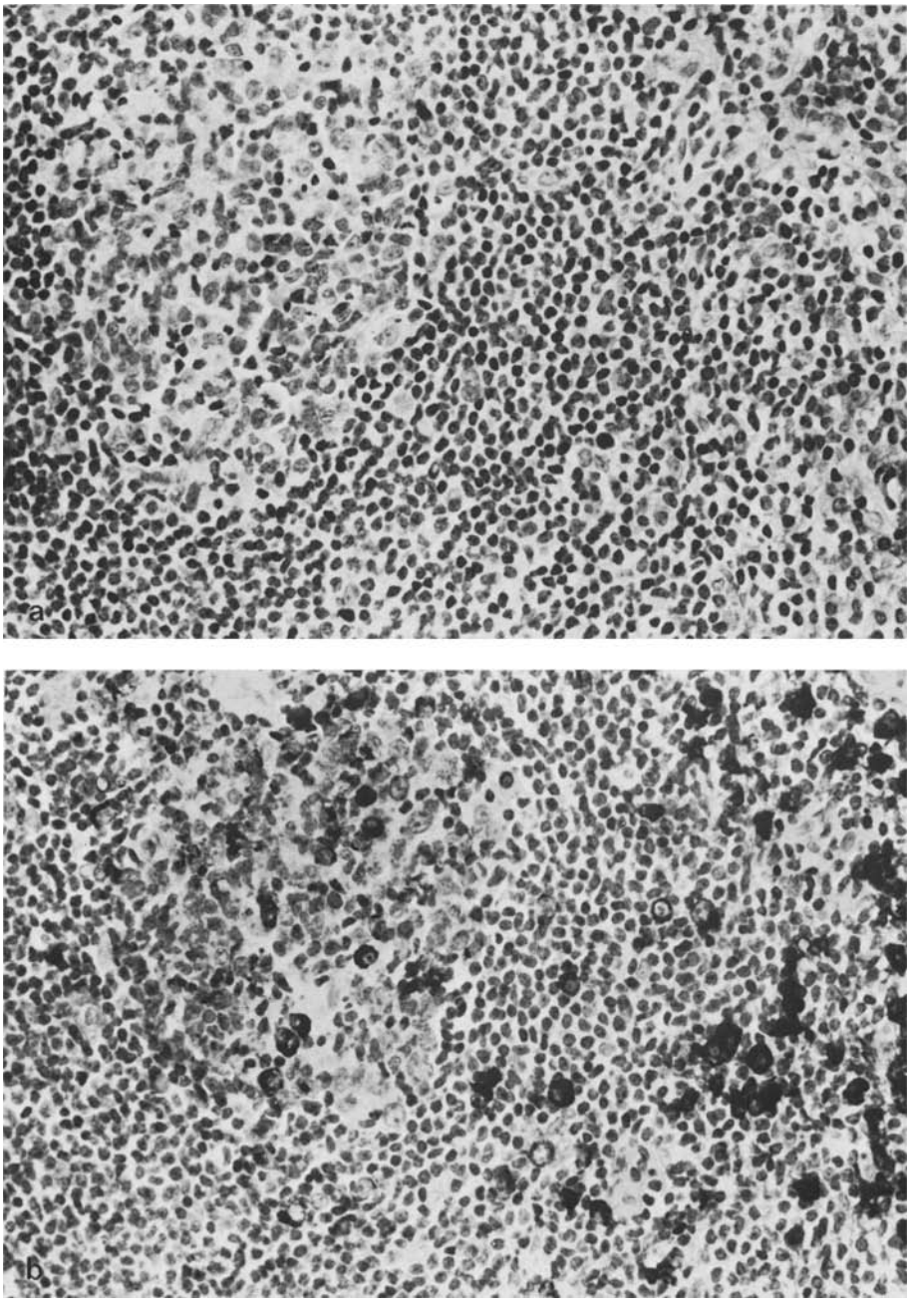

Fig. 1a, b. Case 1. Monoclonal intracytoplasmic immunoglobulin in germinal centre (on the left) and adjoining LP immunocytoma. PAP staining for IgA (*a*) and IgM (*b*). ×350

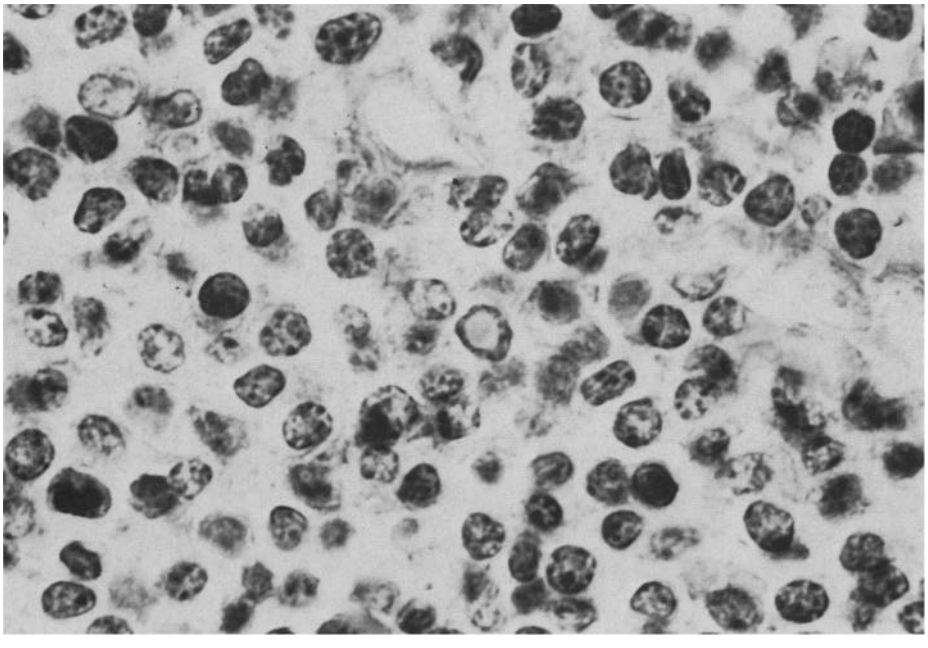

Fig. 2. Case 3. Lymphocytes and lymphoplasmacytoid cells in LP immunocytoma. Note intranuclear PAS-positive inclusion in the centre. PAS. ×1,400

In *case 4* the first tumour excision from the right lacrimal gland was followed by three others, i.e. from both eyes after 5 years and from an unknown site after another 3 years. Histologically, all specimens showed roughly the same picture of a multilobular lymphoid proliferation with well-defined borders (Fig. 3) and additional patchy infiltrates. Each lobule consisted of an active germinal centre surrounded by a broad rim of lymphocytes interspersed with plasma cells. At the periphery of the lobules and in the connective tissue separating them a plasmacytic infiltrate was present. The most recent biopsy differed from the one taken 8 years previously in that the plasmacytic infiltrate was very dense and the germinal centres often showed irregular outlines. In none of the biopsies were PAS-positive inclusions found. Histologically, a diagnosis of pseudolymphoma was made, although in the most recent biopsy LP immunocytoma was also considered.

Immunoperoxidase staining demonstrated both κ and λ light chains in the germinal centres in all specimens; but IgG was constantly more pronounced than IgM, while IgA was virtually absent. In the diffuse areas surrounding the germinal centres and in the peripheral plasmacytic infiltrate a similar pattern of polyclonal light chains and a strong predominance of IgG was found in the initial right-sided biopsy and in the left-sided biopsy after 5 years. In the right-sided biopsy obtained after 5 years and especially in the one obtained after 8 years, however, the peripheral plasmacytic infiltrate appeared to show monoclonal staining for IgG/λ, while a strong predominance of this type was found in the diffuse areas surrounding

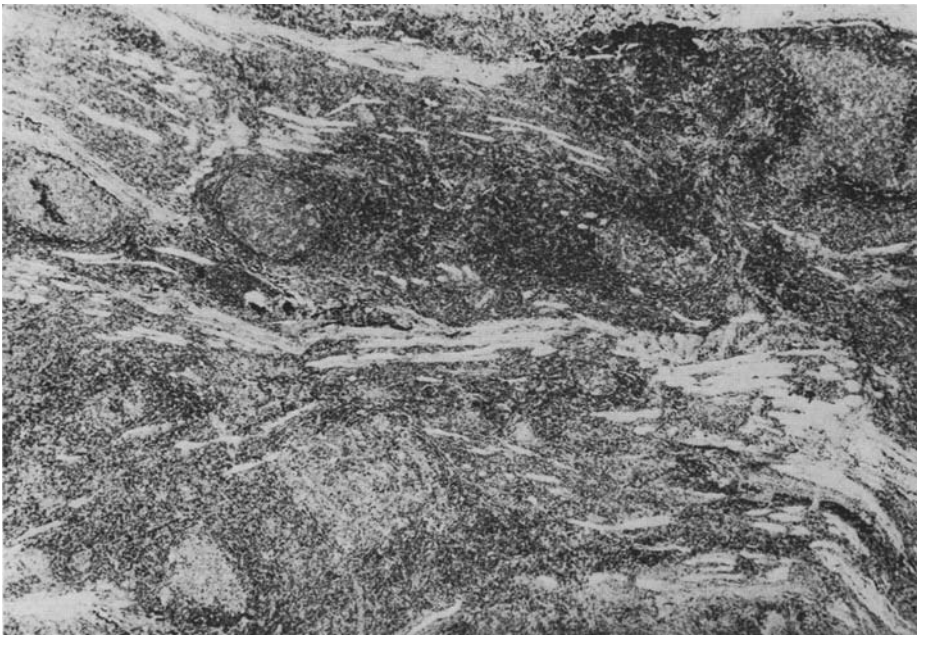

Fig. 3. Case 4, last biopsy. LP immunocytoma with germinal centres. Giemsa. × 35

the germinal centres. In view of these immunological findings, a final diagnosis of pseudolymphoma was made on the first two biopsies and of LP immunocytoma on the most recent one. The third biopsy appeared to represent an intermediate stage.

Discussion

In the differential diagnosis between benign and malignant ophthalmic lymphomas the presence of germinal centres is considered to be a relatively important criterion in favour of the benign forms (Meyer et al. 1970; Garner 1973; Schwarze et al. 1976; Astarita et al. 1980). Among the 4 cases in the present study 3 showed well-developed germinal centres and one showed germinal centre remnants. With immunoperoxidase staining for intracytoplasmic Ig, however, the histologically established diagnoses of LP immunocytoma could be confirmed in 2 cases and LP immunocytoma could be diagnosed in both cases with a doubtful histological picture. These findings thus demonstrate that the presence of germinal centres in ophthalmic lymphomas should be judged critically in order to avoid erroneous benign diagnoses. In this context, it is notable that in our series 8 of 32, or 25% of the immunocytomas showed germinal centres. Furthermore, the importance of immunological studies may be emphasized again (cf. Knowles et al. 1979; Astarita et al. 1980), especially since malignant lymphomas in the eye region are most commonly of low-grade malignancy (Haye et al. 1968;

Schwarze et al. 1976; Kelly et al. 1977; Tewfik et al. 1979; v. Gumberz and Seifert 1980) and are thus difficult to distinguish from pseudolymphomas and reactive processes.

The immunological findings obtained in the present study also provide information concerning the relation between germinal centres and the immunocytomas in which they occur and concerning the development of immunocytoma out of pseudolymphoma. In case 1 the germinal centres expressed the same monoclonality as the surrounding tumour; in cases 2 and 3 the germinal centres were Ig negative; case 4 showed a polyclonal pattern, but with a predominance of IgG in the germinal centres. It is of interest to compare these findings with those in the larger series of immunocytomas developing in myoepithelial sialadenitis in Sjögren's syndrome (Schmid et al. 1982). In that series germinal centres were present in 84% of cases, among which 40% showed a tendency to monoclonal Ig production. In all of the latter cases circumscribed or confluent "proliferation areas" were present, indicating early or manifest lymphoma, which always showed monoclonality of the Ig that was predominant in the germinal centres. These findings, as in the present case 1, suggest a direct relation between the germinal centres and the immunocytoma in which they occur. It may be speculated that the monoclonal plasmacytoid cells, or their precursors, are derived from the germinal centres, which show either monoclonality or strong predominance of the same Ig type.

A polyclonal pattern in the germinal centres was found in cases with sialadenitis without proliferation areas, as in the present case 4. This case is of special interest, because it could be demonstrated that an LP immunocytoma, with monoclonal IgG/λ, developed out of a pseudolymphoma within a period of 8 years. Although the germinal centres in both the pseudolymphoma and the immunocytoma stages appeared to contain both κ and λ light chains, however, IgG was strongly predominant over IgM, while IgA was virtually absent. According to the observations of v. Gumberz and Seifert (1980) IgG is the predominant Ig type produced in sialadenitis associated with Sjögren's syndrome. Histologically, such a diagnosis could not be made in the present case 4 due to the very limited amount of lacrimal gland tissue in the biopsies. The patient apparently suffered from chronic polyarthritis, however, whereas her age, sex and the bilaterality and location of the lesion suggested that the patient had Sjögren's syndrome. Moreover, it is well known (Anderson and Talal 1972; Lennert et al. 1979; Schmid et al. 1982) that in Sjögren's syndrome malignant lymphoma may develop after a long period, and this change is accompanied by a switch from hyper- to hypogammaglobulinaemia (Anderson and Talal 1972; v. Gumberz and Seifert 1980). It may thus be assumed that in the present case 4 the first biopsies represented a stage of reactive lymphoproliferation in which a switch to IgG production and suppression of IgM and especially IgA had already occurred. In the subsequent biopsies the development of a malignant lymphoma with monoclonal Ig production (IgG/λ) became apparent, while the germinal centres remained polyclonal with a predominance of IgG. The strong predominance in the germinal centres of the heavy chain type that

became monoclonal in the plasmacytoid cells and plasma cells suggests a relation between the germinal centres and the surrounding immunocytoma similar to case 1.

In cases 2 and 3 the germinal centres were histologically less pronounced than in the other cases and did not display demonstrable Ig production, while the surrounding lymphoid proliferation showed clear monoclonality. In these cases the germinal centres might have represented remnants of an initially reactive process that later transformed into a malignant lymphoma. This suggestion is supported by the polymorphism of the infiltrate in case 3. In both cases it may be speculated that, initially, the germinal centres gave rise to monoclonal plasmacytoid cells, but after the development of an immunocytoma the germinal centres in turn disappeared. This was clearly seen in case 2, in which the germinal centres progressively became less pronounced in successive biopsies.

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