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Distribution pattern of lymphocyte subpopulations in human tonsils as analysed by monoclonal antibodies with double immunoenzymatic labeling

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Summary. The distribution of B- and T-lymphocytes, T-cell subpopulations, including natural killer cells and monocytes/macrophages, was studied in cryostat sections of human tonsils by the avidin-biotin-peroxidase technique using monoclonal antibodies. A double immunostaining procedure was also developed to detect two types of lymphocytes on one single section simultaneously using horseradish peroxidase and alkaline phophatase as labeling enzymes.

The primary follicles and the germinal centers of the secondary follicles were mainly found to be positive for B-cells. T-cells were predominantly localized in the follicular caps and in the interfollicular areas. The ratio of helper/inducer cells to suppressor/cytotoxic cells was in favour of helper T-cells. Both subpopulations were also predominant in follicular caps and interfollicular areas.

The quantity of natural killer cells was very variable and nearly all were localized exclusively in the germinal centers.

Monocytes/macrophages were only seen occasionally in the interfollicular areas. The double-immunoenzymatic labeling was useful for the visualization of combinations of antigens, however, without demonstrating the presence of two different surface antigens on one single cell.

Key words: Tonsils – Monoclonal antibodies – Immunohistology – Double labeling – Lymphocyte subpopulations

Introduction

The development of numerous monoclonal antibodies for the determination of different lymphocyte subpopulations makes possible not only a better characterization of the T- and B-cell-distribution in lymphoid tissues by different immunohistological techniques, (Lamelin et al. 1978; Hoffmann-

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Frezer et al. 1981; McMillan et al. 1981; Si et al. 1983) but also detection of two (or more) different surface antigens simultaneously on the same cellular preparation. Double labeling can be achieved by either of two ways. One is the immunofluorescence-technique using fluorescein- or rhodamine-isothiocyanate labeled immunoglobulins (Korsrud and Brandtzaeg 1980) but this method has important disadvantages, e.g. short storage periods of the stained slides and the necessity of special microscopic equipment.

The second method is double immunoenzymatic labeling, either by the use of two different substrates for horseradish peroxidase (PO) conjugated antibodies, e.g. 3-amino-9-ethylcarbazol (AEC) with a red colour reaction product and 4-chloro-1-naphthol with a blue one (Si et al. 1983), or by the use of different enzymes linked to two antibodies, resulting in one antigen being visualized for instance by PO, another by alkaline phosphatase. In the latter case the use of primary antibodies from different species is recommended (Mason et al. 1981). Since monoclonal antibodies from different species are not yet readily available, the double immunoenzymatic labeling requires the time-consuming procedure of developing one antigen-antibody-system completely before revealing the second.

In the present study the simultaneous immunoenzymatic labeling of different lymphocyte surface antigens is described to improve the knowledge of the normal distribution pattern of lymphocyte subpopulations in human tonsils.

Materials and methods

For the characterization of T- and B-cells, T-cell subpopulations, including natural killer cells, monocytes and macrophages we used the ABC- (avidin-biotinylated horseradish peroxidase complex) and APAAP-techniques (alkaline phosphatase-anti alkaline phosphatase complex).

Preparation of tissue sections. Fifteen human palatine tonsils were obtained from patients operated for recurrent tonsillitis at the Department of Oto-Rhino-Laryngology of the University Clinics of Freiburg. Immediately after tonsillectomy aliquots of the tissues were snap-frozen in liquid nitrogen and stored at −70° C until sectioned. The remaining tissues were stored in 0.05 M TBS (tris-buffered saline), pH 7.4 at room-temperature (RT) and subsequently either frozen at −70° C or fixed in PLP (paraformaldehyde-lysine-phosphate buffer), pH 7.4 for at least 2 h, then washed three times in 2% saccharose over night at 4° C and frozen at −70° C.

Cryostat sections were air-dried for 2 h and fixed in acetone for 10 min then stored at −20° C under dessicating conditions until the staining, which was executed within 14 days.

Monoclonal antibodies and antisera. All monoclonal antibodies (mabs) to human T- and B-lymphocytes were obtained commercially. Source, specificity and the appropriate dilutions of the monoclonal antibodies that had been determined in preliminary experiments, are listed in Table 1.

Biotinylated horse anti-mouse IgG and avidin-biotinylated PO complex (ABC) were purchased from Vector Laboratories, Atlanta GmbH, Heidelberg, FRG. The reaction substrates 3,3 diaminobenzidine (DAB) and 4-chloro-1-naphthole (CN) were obtained from Sigma, Taufkirchen, FRG. For the indirect immunoenzymatic labeling rabbit anti-mouse IgG coupled with PO was used (Dakopatts, Hamburg, FRG).

Staining procedure with peroxidase. The cryostat sections (5 μm) were thawed to room temperature under dessicating conditions to prevent condensation. Before staining the sections were

Table 1. Antigen recognition, working dilution, specificity and source of monoclonal antibodies used in the present study

Antibody	Dilution	Clone	Specificity	Source
1. Anti-Leu-1[a]	1:20	L17F12	All T-cells	Becton-Dickinson[b] (Engleman and Warnke et al. 1981a)
2. Anti-Leu-4	1:100	SK7	All T-cells (mitogenic)	Becton-Dickinson (Ledbetter et al. 1981)
3. Anti-Leu-3a	1:500	SK3	T-helper/inducer cells	Becton-Dickinson (Evans et al. 1981)
4. Anti-Leu-3a/3b[a]	1:10	SK3/SK4	T-helper/inducer cells	Becton-Dickinson (Evans et al. 1981, Engleman and Benike et al. 1981)
5. DAKO-T8	1:50	DK25	T-cytotoxic/ suppressor-cells	Dakopatts[c] (Reinherz et al. 1980)
6. Anti-Leu-2a[a]	1:10	SK1	T-cytotoxic suppressor-cells	Becton-Dickinson (Evans et al. 1981, Engleman and Benike et al. 1981)
7. Anti-Leu-7	1:100	HNK-1	Large granular lymphocyte (LGL's) including natural killer-cells (NK-cells)	Becton-Dickinson (Abo and Balch 1981)
8. Anti-Leu-11b	1:50	GO 22	LGL's, NK-cells, neutrophils	Becton-Dickinson (Perussia et al. 1984)
9. OKT 10	1:100		Hematopoietic stem cells, activated T- and B-cells, circulating null-cells[d]	Ortho-Diagnostic[e] (Cotner et al. 1981, Cotner et al. 1981a)
10. Dako-pan B	1:50	TO 15	Majority of B-cells	Dakopatts (Stein et al. 1982)
11. Anti-Leu-M3	1:100	MΦP9	Monocytes/ Macrophages	Becton-Dickinson (Dimitriu-Bona et al. 1983)
12. Anti-HLA-DR[a]	1:10	L243	B-cells, monocytes/macrophages, interdigitating	Becton-Dickinson (Lampson et al. 1980)

[a] Reagents from T-cell panel for immunopathology Becton-Dickinson Kit Order No. 95-1001
[b] Becton-Dickinson GmbH, Heidelberg (FRG)
[c] Dakopatts GmbH, Hamburg (FRG)
[d] Appears to be a preferential activity for immature T-cells
[e] Ortho Diagnostic GmbH, Heidelberg (FRG)

washed in 0.04 M PBS (phosphate-buffered saline), pH 7.4 or TBS, pH 7.4. A modification of the avidin-biotin-method described by Hsu et al. (1981) was then used:

1.) The tissue sections were overlayed with the appropriately diluted primary monoclonal antibody and incubated for 30 min in a humidified chamber at 37° C.

2.) The sections were incubated with the second biotinylated horse anti-mouse IgG, diluted 1:100 in PBS for 30 min at RT.

3.) The avidin-biotinylated peroxidase complex (ABC) was achieved by mixing 8 µl biotinylated PO and 8 µl Avidin in 1 ml PBS, for 30 min at RT before use. The tissue sections were incubated with ABC for 45 min at RT.

4.) The PO was visualized by incubation with DAB in 0.06% hydrogen peroxide in PBS for 8–10 min or CN in 0.06% hydrogen peroxide in TBS for 15 min.

5.) The brown colour of the DAB-reaction product was intensified by 0.5% copper sulfate in PBS.

After each of the first five steps the sections were washed three times in PBS for 10 min.

6.) The sections were counterstained with Mayer's Haemalaun before mounting in Vitro-Clud (Langenbrinck, Emmendingen, FRG).

Negative controls consisted of the application of mouse ascites-fluid from mice, which were injected with myeloma cells (SP2/0-AG 14) or the supernatant of a SP2/0-AG cell-suspension or PBS instead of the specific monoclonal antibodies to check the specificity of the reaction and the activity of endogenous peroxidase. Blocking of endogenous peroxidase activity was tried by the use of 0.07% HCl in absolute ethanol or 0.5% hydrogen peroxide and methanol before staining.

For the indirect method the PO-coupled rabbit anti-mouse IgG was used for incubation instead of the biotinylated anti-mouse IgG and the DAB-reaction was subsequently developed.

Staining procedure for double immunoenzymatic labeling. The simultaneous staining of different lymphocyte surface antigens was obtained by a modification of the double immunoenzymatic labeling technique using alkaline phosphatase and peroxidase as described by Mason et al. (1978). The staining reaction using the PO-ABC-technique was developed for one surface antigen (e.g. T8) with CN or DAB and without counterstaining. An other, for instance the Leu-3a reactive antigen, was subsequently stained with the immune complex of alkaline anti-alkaline phosphatase (APAAP-complex) from Dianova, Hamburg, FRG (Cordell et al. 1984):

1.) Incubation with the appropriately diluted monoclonal antibody for 30 min in a humidified chamber at 37° C.

2.) The slide was then incubated with rabbit anti-mouse IgG, diluted 1:100, and functioning as a bridge-antibody for 30 min at RT.

3.) The APAAP-complex, diluted 1:150 in TBS was used for incubation for 45 min at RT.

4.) Step 2.) and 3.) were repeated twice with an incubation-time of 15 min per step.

5.) The alkaline phosphatase substrate was incubated for 30 min at RT.

The reaction-substrate for alkaline phosphatase was prepared as follows: 0.5 ml of a 4% fuchsin-solution, diluted in 2 M HCl, was mixed with 1.25 ml of a freshly prepared 4% sodium nitrite-solution and added to 8 ml of a stock solution of 5 mg naphthol-AS-BI-phosphate and 100 µl dimethylformamide in 20 ml 0.2 M TBS, pH 9.0 containing 5 mg levamisole for the inhibition of endogenous alkaline phosphatase (Ponder and Wilkinson 1981). This substrate had to be filtered twice before use. The sections were washed three times in PBS after each step and subsequently mounted in Fluoprep (Bio Merieux, Nürtingen, FRG).

Figure 1 illustrates the double immunoenzymatic labeling procedure.

Results

Positive staining of cell membranes of T- and B-lymphocytes was obtained with all twelve monoclonal antibodies used on the 75 acetone-fixed cryostat sections of all 15 frozen tonsil-tissues. The results are summarized in Table 2. From the brown ring of the specific PO-DAB of the membrane staining the positive cells could easily be distinguished from the very few cells in

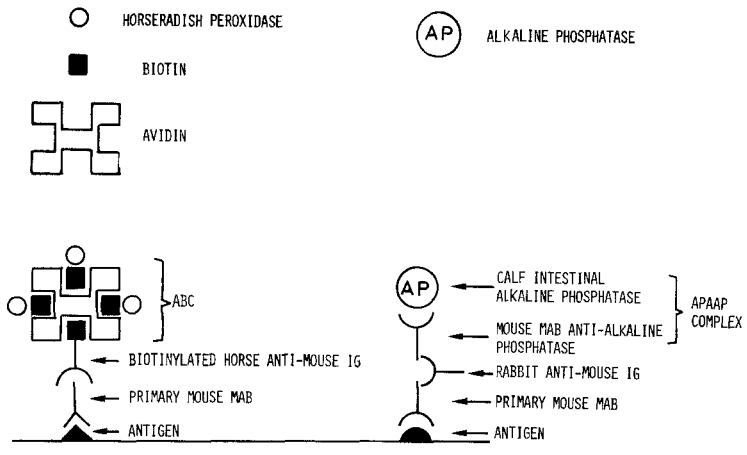


Fig. 1. Schematic illustration of the double immunoenzymatic labeling of two different surface-antigens with two different mouse monoclonal antibodies on one single tissue-section. Note that the first labeling with ABC including the development of the peroxidase reaction product (not illustrated) must be finished before the other labeling with APAAP can be started

Table 2. Distribution pattern of lymphocyte surface antigens. Results of immunoperoxidase staining with the ABC-technique at fifteen human tonsils

	Germinal center	Follicular cap	Interfollicular area
Anti-Leu-1[a]	++	++	++++
Anti-Leu-4	++	++	++++
Anti-Leu-3a	++	++	++++
Anti-Leu-3a/3b[a]	++	++	++++
DAKO-T-8	+	+	+++
Anti-Leu-2a[a]	+	+	+++
Anti-Leu-7	++	−	+
Anti-Leu-11b[b]	−	−	+
OKT-10	++	−	++
DAKO-Pan-B	++++	++++	++
Anti-Leu-M3	++	−	++
Anti-HLA-DR[a]	++++	++++	++++

[a] Monoclonal antibody was tested on three different tonsils only
[b] Results were confirmed by use of alkaline phosphatase (APAAP-) technique

the negative controls which had a positive (brown) cytoplasmatic staining, due to endogenous peroxidase. The only exception was seen with the Leu-11b mab. If this mab was used the results were confirmed using the APAAP-technique. Endogenous activity of alkaline phosphatase was never observed. However, blocking of endogenous peroxidase activity was found to have a deleterious effect on the surface antigens of lymphocytes.

The intensity of the colour reaction obtained with the ABC-technique was much stronger than that of the indirect immunoperoxidase technique. Also the unfixed material gave superior staining results when compared to the material fixed in PLP before staining.

All controls were negative using the ABC-technique except for very few cells exhibiting endogenous peroxidase activity, but they were absolutely negative when alkaline phosphatase was used as the catalyzing enzyme.

Distribution pattern of T-cell subpopulations

The germinal centers and the caps of the secondary follicles as well as the primary follicles mainly contained B-cells, as identified by the mab DAKO-PAN-B. Some B-cells were also found in the T-cell areas. The intensity of the mab PAN-B-staining was much weaker than that obtained with all other mabs.

T-cells, identified with mab Leu-1 and mab Leu-4 were found predominantly in the interfollicular area and in the caps of the secondary follicles, some also in the germinal center (see Fig. 2a). Very few T-cells were observed in the primary follicles. The labeling of the cells with mab Leu-4 gave better results when compared with the staining by mab Leu-1, because a single positive cell could be clearly separated from a negative one. This is not possible in every case of labeling with mab Leu-1.

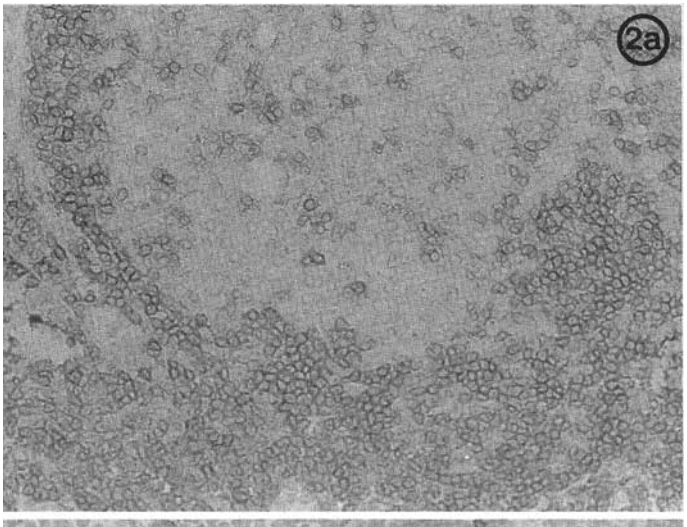
Helper/inducer cells, identified with mab Leu-3a and -3a/3b, were mostly seen in the interfollicular area, but also in the germinal center and in the follicular cap. The overall positivity was only slightly less than that obtained by the PAN-T-mabs. As expected mab Leu-3a/3b recognized more helper cells than Leu-3a alone. Both antibodies seemed to label not only antigens of helper-T-cells but also antigens of the monocyte-macrophage series.

The results for the suppressor-cytotoxic cell staining, when the mabs DAKO-T-8 and Leu-2a were used, were similar. There were clearly fewer suppressor-cytotoxic cells in the interfollicular areas than helper/inducer cells and very rare positive cells were seen in the follicular cap and the germinal center of secondary follicles (see Fig. 2b).

Natural killer cells were detected with the mabs Leu-7 and Leu-11b. The staining results with mab Leu-7 showed labeled cells predominantly in the germinal centers. The quantity of them varied from relatively few in some tissue-samples to numerous ones in others (see Fig. 2c). Some mab Leu-7 positive cells were also found in the interfollicular area, but never as many as in the germinal centers.

Mab Leu-11b only labeled very few cells in the interfollicular areas. Mab Leu-11b positive cells could only be determined with the APAAP-technique due to clear cut negative controls using alkaline phosphatase. Usually mab Leu-11b positive cells were found in small clusters. Mabs OKT-10 and Leu-M3 labeled some cells in the interfollicular areas and in the germinal centers. Rarely mab OKT-10 positive cells were also found

Fig. 2a–c. Immunohistological staining of different lymphocyte subpopulations, counterstaining with Mayer's Haemalaun. **a** T-cells (Leu-4) are mainly found in the interfollicular area (×170). **b** Suppressor/cytotoxic cells (T-8) also predominate in that area (×170). **c** Numerous NK-cells (Leu-7) are stained in the germinal center of a secondary follicle (×70)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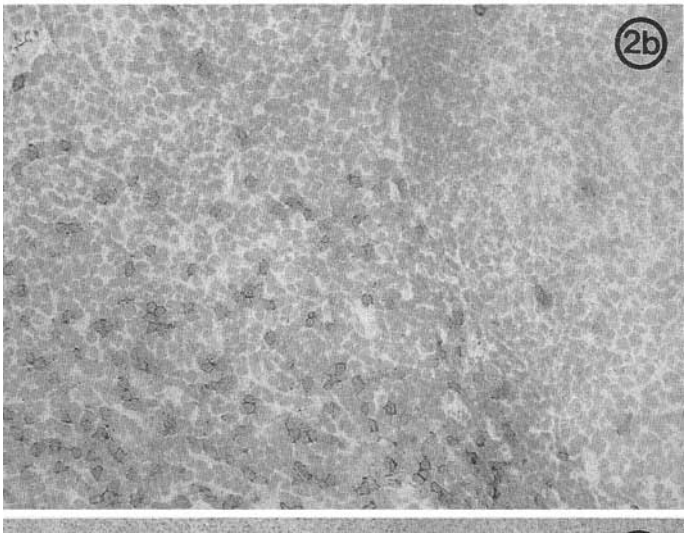
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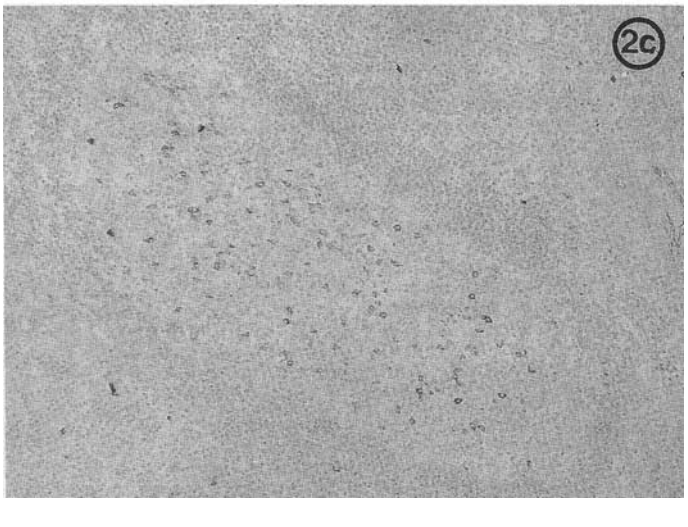
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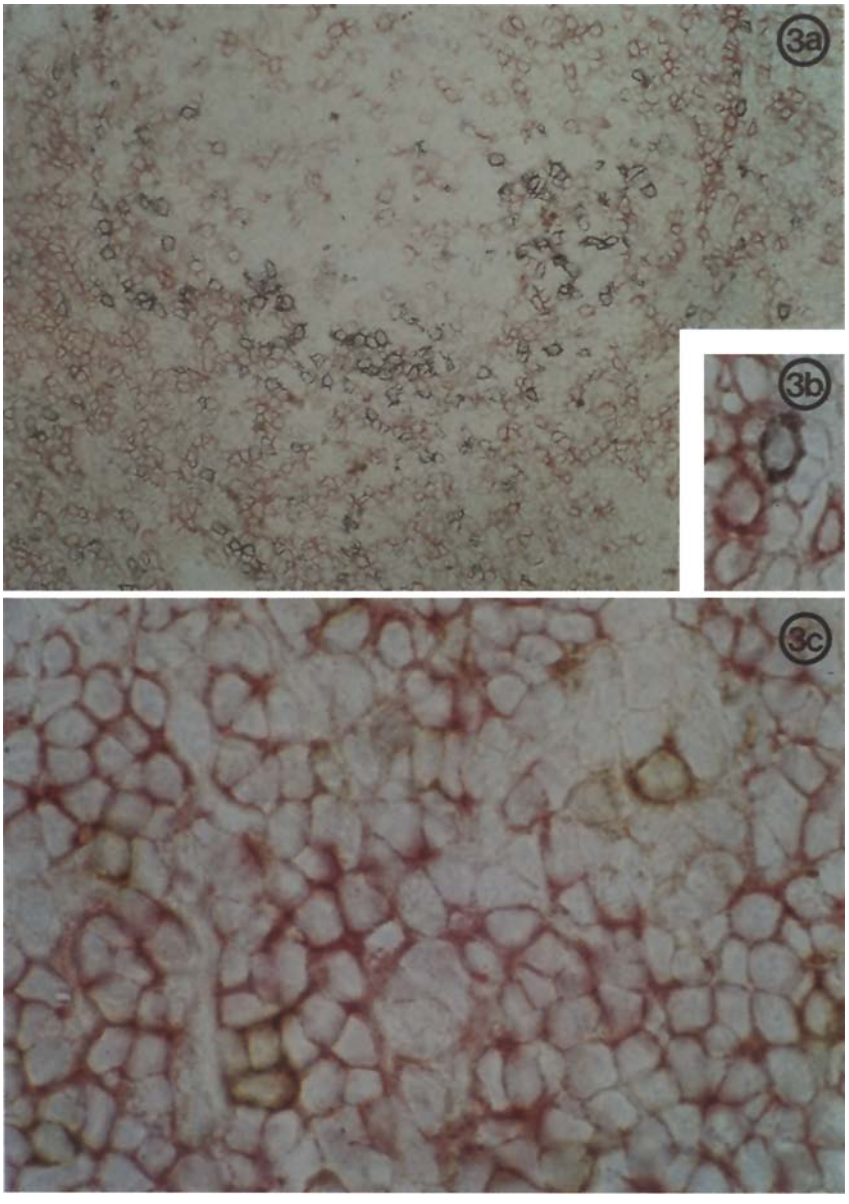

Fig. 3a–c. Double immunoenzymatic labeling without counterstaining. **a** Suppressor/cytotoxic cells (DAKO T-8), which are stained with the PO-ABC-technique and CN as enzyme substrate (blue-black membrane staining) are fewer than helper/inducer cells, which are stained with the APAAP-technique (red membrane staining) (× 170). **b** (× 670). **c** The same pair of antigens are stained, but DAB was used instead of CN (× 670)

in the follicular cap. Mab HLA-DR labeled nearly every cell of germinal centers, follicular caps and primary follicles. The density of positive cells was only slightly less in the interfollicular area where 60–80% positive cells were found.

Double immunoenzymatic labeling

Satisfactory results were obtained with the double labeling-technique in some combinations of monoclonal antibodies used. There was no mixture of colours of the enzyme products when the sections were stained with the mabs DAKO-T8 and Leu-3a, Leu-7 and Leu-11b or Leu-M3 and OKT-10, provided that the first labeling had been finished including the development of the enzyme reaction product before the other labeling was started. Double labeling with DAKO-T8 and Leu-3a depended on the sequence: If DAKO-T8 was used for the first labeling with PO-ABC and Leu-3a for the second labeling with AP no mixture resulted of the two staining products obtained (see Fig. 3a and b). When the same antigens were labeled in reverse order some cells adopted a more or less violet surface staining instead of a clear blue or red one due to a mixture of both reaction products. This kind of a mixed colour was also obtained when DAKO-T8 and Leu-4 were used.

Using PAN-B for the first labeling and Leu-4 for the second was better than the reverse order due to the appearance of a mixture of the reaction products in the latter case.

Discussion

By using monoclonal antibodies reactive with different surface antigens of mononuclear cells we have characterized the localization and distribution of B- and T-cells, T-cell subpopulations and monocytes-macrophages. We obtained our results with the ABC-technique, which we found to be much more sensitive than the indirect immunoperoxidase-technique, which corresponds to the findings of Hsu, Raine and Fanger (1981 and 1981a). The antigenic reactivity of surface markers of lymphocytes with monoclonal antibodies is better preserved on acetone-fixed than on PLP-fixed cryostat sections of frozen tissues and better than on paraffin embedded tissues, which had been fixed in formalin. However, Stein et al. (1984) described results of equal or greater intensity of labeling reactions when using material that had been freeze-dried before being embedded in paraffin, than with cryostat sections.

In agreement with the studies of McMillan et al. (1981) and Si et al. (1983 and 1983a) we found that blocking of endogenous peroxidase activity with a methanol-hydrogen peroxide mixture damaged the lymphocyte surface antigens.

In order to characterize better the T-cell subpopulations we developed a double immunoenzymatic labeling-technique, which allowed us to detect two different surface antigens on one histological section simultaneously and demonstrated that this method is not suitable for the recognition of two antigens on one single cell.

Using the mabs PAN-B, Leu-1 and Leu-4, we have demonstrated that T-cells are localized predominantly in the interfollicular areas and in the follicular caps. Very few are found in the primary follicles. However, T-cells could also be found in the germinal centers in contrast to B-cells, which

predominated in the germinal centers, follicular caps and in the primary follicles.

For an unknown reason the intensity of the membrane staining with mab PAN-B was less compared with the other monoclonal antibodies. This did not depend on the concentration of the mabs used.

Our results of the distribution pattern of T- and B-cells including the T-cell subsets in the tonsils confirm the findings of other authors, who used polyclonal antisera (Lamelin et al. 1978) or monoclonal antibodies (McMillan et al. 1981; Si et al. 1983; Gaudecker et al. 1984). According to the presumption of Gaudecker and her colleagues (1984) the presence of some T-helper/inducer cells within the B-cell-areas (germinal centers) may play a role in the differentiation and maturation of B-cells or in the T- B-cell cooperation. Poppema and his group (1981) obtained similar results in their study of the distribution of T-cell subsets in lymph nodes, and they also discussed the presence of T-helper/inducer cells as being necessary for the induction of B-cell differentiation.

The interpretation of the results obtained with the mabs Leu-3a and -3a/3b is limited, because of the cross-reaction of these antibodies with monocytes/macrophages and other intercellular structures. This observation confirms earlier findings of Wood et al. (1983).

The presence and function of NK-cells in lymphoid tissues has raised attention when it became possible to determine this special subpopulation by monoclonal antibodies such as Leu-7 (HNK-1) (Si and Whiteside 1983a; Gaudecker et al. 1984). Since this mab also detects large granular lymphocytes (LGL's), (Abo and Balch 1981) besides numerous small lymphocytes, the few larger cells detected in the interfollicular area could therefore be LGL's. The observation that the small Leu-7 positive cells were concentrated in the B-cell areas suggests the possibility of either an involvement of NK-cells in the regulation of B-cells or an activation of NK-cells by B-cells. Banerjee and Thibert (1983) and Gaudecker and her group (1984) described the mab Leu-7 positive cells in the germinal centers (where we also saw small lymphocytes) as functionally inactive precursors of NK-cells.

The mab Leu-11b only detected very few cells in the interfollicular areas, which we suppose to be LGL's. There were never Leu-11b positively labeled cells in the germinal centers. We conclude that this mab either does not detect any surface antigen, expressed on Leu-7 positive cells, or this mab is not very suitable for immunohistology.

The mabs Leu-M3 and OKT-10 labeled fewer cells in the interfollicular areas and in the germinal areas, than there were actually present, e.g. macrophages often remained unstained. The use of these mabs, however, was difficult because of unreliable results obtained with the immunohistological techniques employed in our study. Likewise the mab anti-HLA-DR, which stained nearly every cell, turned out to be not very useful in this context, because only in the interfollicular area were there 20–40% unlabeled cells.

By using a double immunoenzymatic labeling we stained two surface antigens successively on one section, firstly to visualize a pair of antigens simultaneously and to study their quantitative ratio and secondly to find

out whether two antigens are expressed on a single cell. We demonstrated that the success of a double immunoenzymatic labeling depended on two conditions: The two antigens must be localized on different cells with a few unstained cells between them and the labeling must start with the surface antigen expressed on the least numerous cells followed by staining for the antigen present most abundantly.

If this order was not followed a mixed colour was observed but we could not distinguish whether it was due to an antibody interaction of the second labeling (rabbit anti-mouse) with the mouse monoclonal antibody from the first labeling or whether the two antigens in question were actually expressed on the surface of one single cell (see Fig. 1). We believe, however, that the steric hindrance of the big complexes of the enzyme conjugated third layer (ABC and APAAP) and the reaction-products do not allow the labeling of two different antigens on single cells or cells lying side by side.

We prefer this explanation of our mixed colour results by antibody interactions which occurred only if the antigen for the second labeling was localized on fewer cells than that for the first staining. Two antigens on one section could be demonstrated simultaneously in some cases. There was no evidence for the localization of two antigens on the surface of one single cell.

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References

Abo T, Balch CM (1981) A differentiation antigen of human NK and K cells identified by a monoclonal antibody (HNK-1). J Immunol 127:1024–1029

Banerjee D, Thibert RF (1983) Natural killer-like cells found in B-cell compartments of human lymphoid tissues. Nature 304:270–272

Cordell JL, Falini B, Erber WN, Ghosh AK, Abdulaziz Z, McDonald S, Pulford KAF, Stein H, Mason DY (1984) Immunoenzymatic labeling of monoclonal antibodies using immune complexes of alkaline phosphatase and monoclonal anti-alkaline phosphatase (APAAP complexes) J Histochem Cytochem 32:219–229

Cotner T, Mashimo H, Kung PC, Goldstein G, Strominger JL (1981) Human T cell surface antigens bearing a structural relationship to HLA antigens. Proc Natl Acad Sci USA 78:3858–3862

Cotner T, Hemler M, Strominger JL (1981a) Human T cell proteins recognized by rabbit heteroantisera and monoclonal antibodies. J Immunopharmacol 3:255–268

Dimitriu-Bona A, Burmester GR, Waters SJ, Winchester RJ (1983) Human mononuclear phagocyte differentiation antigens. I. Patterns of antigenic expression on the surface of human monocytes and macrophages defined by monoclonal antibodies. J Immunol 130:145–152

Engleman EG, Benike CJ, Glickman E, Evans RL (1981) Antibodies to membrane structures that distinguish suppressor/cytotoxic and helper T lymphocyte subpopulations block the mixed leukocyte reaction in man. J Exp Med 153:193–198

Engleman EG, Warnke R, Fox RI, Dilley J, Benike CJ, Levy R (1981a) Studies of a human T lymphocyte antigen recognized by a monoclonal antibody. Proc Natl Acad Sci USA 78:1791–1795

Evans RL, Wall DW, Platsoucas CD, Siegal FP, Fikrig SM, Testa CM, Good RA (1981) Thymus-dependent membrane antigens in man: Inhibition of cell-mediated lympholysis by monoclonal antibodies to T_{H2} antigen. Immunology 78:544–548

Gaudecker von B, Pfingsten U, Müller-Hermelink HK (1984) Localization and characterization of T-cell subpopulations and natural killer cells (HNK 1^+ cells) in human tonsilla palatina. Cell Tissue Res 238:135–143

Hoffmann-Fezer G, Löhrs U, Rodt HV, Thierfelder S (1981) Immunohistochemical identification of T- and B-lymphocytes delineated by the unlabelled antibody enzyme method III. Topographical and quantitative distribution of T- and B-cells in human palatine tonsils. Cell Tissue Res 216:361–375

Hsu SM, Raine L, Fanger H (1981) Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: A comparison between ABC and unlabeled antibody (PAP) procedures. J Histochem Cytochem 29:577–580

Hsu SM, Raine L, Fanger H (1981a) The use of antiavidin antibody and avidin-biotin-peroxidase complex in immunoperoxidase technics. Am J Clin Pathol 75:816–821

Korsrud FR, Brandtzaeg P (1980) Quantitative immunohistochemistry of immunoglobulin and J chain producing cells in human parotid and submandibular salivary glands. Immunology 39:129–140

Lamelin JP, Thomasset N, Andre C, Brochier J, Revillard JP (1978) Study of human T and B lymphocytes with heterologous antisera. III. Immunofluorescence studies on tonsil sections. Immunology 35:463–469

Lampson LA, Levy R (1980) Two populations of Ia-like molecules on a B cell line. J Immunol 125:293–299

Ledbetter JA, Evans RL, Lipinski M, Cunningham-Rundles C, Good RA, Herzenberg LA (1981) Evolutionary conservation of surface molecules that distinguish T lymphocyte helper/inducer and cytotoxic/suppressor subpopulations in mouse and man. J Exp Med 153:310–323

Mason DY, Sammons R (1978) Alkaline phosphatase and peroxidase for double immunoenzymatic labelling of cellular constituents. J Clin Pathol 31:454–460

Mason DY, Stein H, Naiem M, Abdulaziz Z (1981) Immunohistological analysis of human lymphoid tissue by double immunoenzymatic labelling. J Cancer Res Clin Oncol 101:13–22

McMillan EM, Wasik R, Everett MA (1981) Identification of T-lymphocytes and T-subsets in human tonsil using monoclonal antibodies and the immunoperoxidase technic. Am J Clin Pathol 76:737–744

Perussia B, Trinchieri G, Jackson A, Warner NL, Faust J, Rumpold H, Kraft D, Lanier LL (1984) The Fc receptor for IgG on human natural killer cells: phenotypic, functional, and comparative studies with monoclonal antibodies. J Immunol 133:180–189

Ponder BA, Wilkinson MM (1981) Inhibition of endogenous tissue alkaline phosphatase with the use of alkaline phosphatase conjugates in immunohistochemistry. J Histochem Cytochem 29:981–984

Poppema S, Bhan AK, Reinherz EL, McCluskey RT, Schlossman SF (1981) Distribution of T cell subsets in human lymph nodes. J Exp Med 153:30–41

Reinherz EL, Kung PC, Goldstein G, Schlossman SF (1980) A monoclonal antibody reactive with the human cytotoxic/suppressor T cell subset previously defined by a hetero-antiserum termed TH_2. J Immunol 124:1301–1307

Si L, Roscoe G, Whiteside TL (1983) Selective distribution and quantitation of T-lymphocyte subsets in germinal centers of human tonsils. Arch Pathol Lab Med 107:228–231

Si L, Whiteside TL (1983a) Tissue distribution of human NK cells studied with Anti-Leu-7 monoclonal antibody. J Immunol 130:2149–2155

Stein H, Gerdes J, Mason DY (1982) The normal and malignant germinal center. Clin Haematol 11:531–559

Stein H, Gatter KC, Heryet A, Mason DY (1984) Freeze-dried paraffin-embedded human tissue for antigen labelling with monoclonal antibodies. Lancet 14:71–73

Wood GS, Warner NL, Warnke RA (1983) Anti-Leu-3/T 4-antibodies react with cells of monocyte/macrophage and langerhans lineage. J Immunol 131:212–216

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