

Use of anti-receptor antibodies to focus T-cell activity

Uwe D. Staerz and Michael J. Bevan

The T-cell receptor dictates that T cells recognize foreign antigen in the context of self major histocompatibility complex (MHC) encoded molecules. Cells which are recognized as foreign by the T-cell system are efficiently rejected by direct cytotoxicity or in a lymphokine-mediated way. Meanwhile, antibodies recognize native antigen and can be made available reproducibly and in quantity. Here, Uwe Staerz and Michael Bevan discuss the use of hybrid antibodies, composed of anti-receptor/anti-X, to confer the specificity of antibody to a T-cell response.

Thymus-derived (T) lymphocytes are specific for foreign antigen (or a 'processed' fragment of the antigen) recognized in association with a particular self major histocompatibility complex (MHC) encoded surface marker. This phenomenon is MHC restriction of T-cell specificity. Most cytotoxic T cells (CTL) recognize antigen plus class I MHC molecule, while most helper/inducer T cells recognize antigen plus class II molecule. Although there are CTL which recognize class II alloantigens, or T1a/Qa encoded class I-like antigens, and while some helper T cells recognize the undefined product of the M1s locus, there are no examples of T cells which recognize foreign antigens such as sheep red blood cells, or keyhole limpet haemocyanin, or viral antigens in a non-MHC-restricted fashion. T-cell specificity for antigen plus MHC may be attributed to the particular pair of α and β chains of the T-cell receptor expressed at the surface, though other T-cell surface structures may be involved. The α and β chain genes of the T-cell receptor are put together in T cells by rearrangement of V, D, J and C region gene segments as with the heavy and light chain genes in B lymphocytes. The disulphide-bonded $\alpha\beta$ heterodimer is physically associated on the T-cell surface with the clonally invariant T3 polypeptides. The T4 molecule on T cells may aid in the binding of class II molecules, and T8 may bind class I molecules¹.

Selective T-cell activation always involves the recognition of MHC molecules, except in one special and entirely artificial instance which is the basis of this review. A monoclonal antibody (mAb) which is specific for a determinant on the T-cell receptor $\alpha\beta$ T3 complex can also be the target structure for T-cell activation and for the delivery of effector function such as cell lysis or lymphokine release. As we will describe, this process is independent of the MHC molecules of the target or T cell, and when an anti-receptor antibody is physically linked to a different antibody, then the specificity of the second antibody is conferred to the T cell. Since a variety of mAb against the T-cell receptor complex are available, and since one can anticipate the availability of mAb to any viral, differentiation, or tumor-associated antigen, then it is reasonable to explore the feasibility of using bispecific or hybrid antibodies to focus effector T cells to the site of infections or tumors. The superiority of T cells over antibody in rejecting unwanted tissue is well-

accepted; for example, in rejecting tissue allografts and in combating some viral infections. Bispecific, anti-receptor/anti-X antibodies offer a way of coupling T-cell effector function with the specificity and availability of mAb.

Hybridoma cells as targets for T cells

B-cell hybridomas which secrete large amounts of antibody also express copies of the antibody at the cell surface, probably because a small fraction of it is synthesized with the membrane attachment region on the heavy chains. This surface Ig can be the site of T-cell attack. Two groups have reported that hybridomas secreting antibody specific for idiotype determinants on the antigen plus MHC receptor of CTL clones were sensitive targets for lysis by the corresponding CTL. Lysis was idiotype-specific and did not appear to involve the class I MHC molecules of the target hybridoma^{2,3}. Murine hybridomas producing anti-T3 antibodies also serve as targets for any human CTL clone⁴. Hybridoma lines which produce antibodies recognizing other surface molecules of the CTL, such as class I, Lfa-1 or T4, and which allow conjugate formation with the CTL, did not serve as lysable targets.

mAb coupled cells as targets

The anti-receptor mAb need not be incorporated in the target cell membrane to provide a site for delivery of a lethal hit. When mAb specific for an idiotype determinant on the $\alpha\beta$ heterodimer, or for an allotypic determinant on the β chain of the receptor are chemically coupled to cells, these cells become targets for CTL lysis^{5,6}. We explored this system to establish the minimum requirements, at the target cell level, for lysis to proceed. The R1.TL⁻ lymphoma-cell line which is deficient in β_2 -microglobulin and thus expresses no class I

Table 1. Antibody-directed lysis of target cells covalently coupled with anti-receptor idiotype or allotype mAb

Target Cell	% specific lysis by CTL clones	
	OE4	G4
P815 (H-2 ^d)	85	60
R1.TL ⁻ (H-2 ^b)	-1	-1
R1.TL ⁻ -F23.1	46	-1
R1.TL ⁻ -F9	1	21
SRBC	0	ND
SRBC-F23.1	34	ND

The mAb F23.1 (anti-allotype) and F9 (anti-idiotype) were reacted with the cross-linking reagent SPDP and then bound covalently to ⁵¹Cr-labelled target cells. Both CTL clones, OE4 and G4, are from H-2^b mice and are specific for H-2^d antigens. OE4 is recognized by mAb F23.1, and G4 is recognized by mAb F9. They were used as an effector: target ratio of 3:1 in a 4 h assay.

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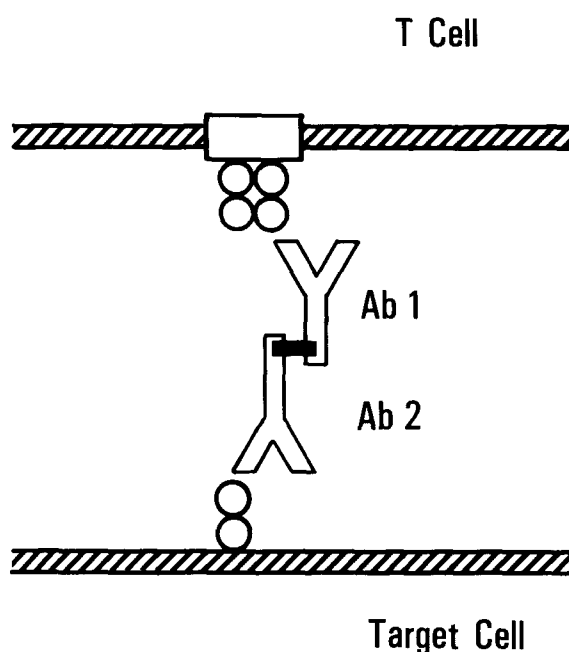


Fig. 1. To direct the activity of a T cell, antibody 1 must recognize a component of the $\alpha, \beta, T3$ complex, while antibody 2 may be directed at any determinant expressed at the target surface.

surface molecules (and no class II molecules) was sensitive to anti-idiotypic or anti-allotypic mAb-directed lysis^{5,7}. Furthermore, sheep or human erythrocytes chemically coupled with antibody also serve as target cells for CTL (Table 1). Again, in this system mAb with specificity for other T-cell surface structures did not act as target sites for the delivery of a lethal hit.

Heteroconjugated antibodies allow specific targeting

The realization that anti-receptor antibody on a cell surface could act as a trigger for the delivery of effector T cell activity led to the notion that bispecific antibodies in which one of the specificities is for the T-cell receptor could provide a means to use antibody combining sites to direct T-cell activity. One illustration of this which does not involve hybrid antibodies is the demonstration that an anti-T-cell receptor antibody of a particular subclass can direct CTL to lyse any Fc receptor-bearing cell. In this case, the antibody Fab region binds the T-cell receptor and the Fc portion of the antibody fixes to the target⁸⁻¹⁰.

Initially, in the production of heteroconjugated mAb the cross-linking reagent N-succinimidyl 3-(2-pyridyldithiol) propionate (SPDP) was used to cross-link the two different antibodies. This heterobifunctional cross-linker reacts with the free $-NH_2$ or $-SH$ groups of proteins in physiological saline. The antibodies, which contain no free $-SH$ groups, were modified so that they bore 1-3 PDP groups per 7S molecule. One of the modified antibodies was mildly reduced to create $-SH$ groups on the linker, and the two antibodies when mixed together formed covalent heteroconjugates. Dimers of 7S were separated from monomers and higher forms by chromatography. We reported experiments in which an anti-Thy 1.1 mAb was cross-linked to an anti-idiotypic mAb or to an anti- β -chain allotypic mAb⁶. Other workers made conjugates containing an anti-T3

antibody and anti-MHC mAb, or anti-TNP mAb^{11,12}, or anti-immunoglobulin idiotype¹³. In each case the conjugates worked efficiently and specifically in allowing a CTL recognized by the anti-receptor mAb to lyse any target cell recognized by the second mAb. This is shown schematically in Fig. 1. Using the same conjugation procedure, we have shown that virus-infected cells can be specifically targeted for CTL lysis: anti-receptor/anti-G protein conjugates target VSV-infected EL4 cells⁷, and conjugates of anti-receptor and anti-haemagglutinin or anti-nucleoprotein mAb target influenza-infected cells for lysis (unpublished). Furthermore, human melanoma cells can be lysed by murine CTL in the presence of an anti-receptor-anti-melanocyte mAb conjugate (unpublished work with R.A. Reisfeld).

Bispecific, bivalent antibodies can also be prepared in a test tube in the form of recombinant $F(ab')_2$ fragments. Pepsin was used to prepare $F(ab')_2$ fragments from two murine IgG2a antibodies - F23.1 (anti-receptor allotype) and 19E12 (anti-Thy 1.1). The purified fragments were reduced and the column-purified Fab' monomers were combined and reoxidized. Such recombinant $F(ab')_2$ fragments also allowed an F23.1⁺ CTL clone to lyse any target expressing the Thy 1.1 determinant¹⁴.

A hybrid hybridoma cell line

By fusing two hybridoma cell lines, both of which secrete an IgG2a antibody, we obtained a hybrid hybridoma cell line which secretes a fraction of its total Ig in the form of a bispecific hybrid antibody¹³. This hybrid antibody is extremely efficient in focusing effector T-cell activity. The parents of this cell line were the F23.1 hybridoma secreting a murine IgG2a specific for an allotypic determinant on a family of murine β chains expressed by approximately 25% of T cells, and the 19E12 hybridoma secreting an IgG2a anti-Thy 1.1 antibody. To select for hybrid cells, a HAT-sensitive, ouabain-resistant variant of F23.1 was fused with 'wild type' 19E12 (HAT-resistant, ouabain-sensitive) and the fusion products selected for growth in medium containing HAT and ouabain. A stable clone, H1.10.1.6, was isolated which secreted antibodies of both original specificities, anti-receptor and anti-Thy 1.1, as determined by fluorescence-activated cell sorter analysis. Ascites fluid was obtained from mice bearing the H1.10.1.6 hybrid hybridoma, the immunoglobulin isolated on Protein A and fractionated on hydroxylapatite high pressure liquid chromatography (HPLC). The profile of the elution is shown in Fig. 2A. The shaded areas under the three peaks were pooled, concentrated, and rechromatographed under identical conditions (Fig. 2B, C, D). Pools of the major peaks were made as shown and assayed functionally. For this, the CTL clone 0E4 (H-2^b anti-H-2^d, F23.1⁺) was assayed for lysis of S.AKR (H-2^k, Thy 1.1⁺) targets at a 3:1 effector: target ratio in the continuous presence of the H1.10.1.6 immunoglobulin fractions (Fig. 3). Peak I, which corresponds in its HPLC elution position with F23.1, has little targeting activity; Peak II is very active in this assay and is able to cause 30% lysis of the targets at a protein concentration as low as 2 ng/ml; Peak III, which corresponds in its elution position with 19E12, has about 1% the activity of Peak II, which is consistent with some degree of cross-contamination as seen in Fig. 2D. Control experiments showed that for CTL lysis to occur, the T cell must express a receptor recognized by F23.1 and the target cell must express Thy 1.1.

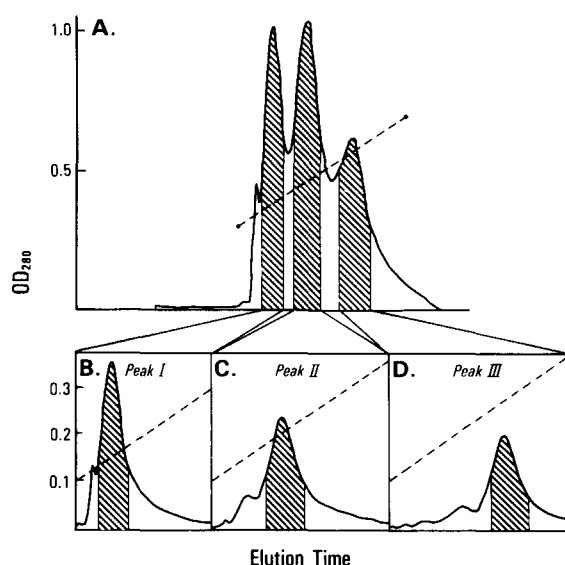


Fig. 2. HPLC fractionation of hybrid antibody. The hybrid hybridoma-cell line H1.10.1.6 was produced by fusing the F23.1 (anti-T-cell receptor) and 19E12 (anti-Thy 1.1) hybridomas. Immunoglobulin was purified from the ascites fluid of mice bearing H1.10.1.6, loaded on a hydroxylapatite HPLC column (Bio-Gel HPHT, Biorad) in 50 mM sodium phosphate buffer, pH 6.8, and eluted with a 50–350 mM gradient. Shaded areas in A were rechromatographed under the same conditions in B, C, and D.

This stable hybrid hybridoma cell line thus provides a constant source of a native, 7S hybrid antibody. The factors that affect the yield of the desired bispecific antibody have been discussed by Milstein and Cuello¹⁵. Briefly, if the rate of production of the two pairs of heavy (H) and light (L) chains is the same, and if the formation of HL pairs shows no homologous or heterologous preference (i.e., if L_1H_1 , L_2H_2 , L_1H_2 , and L_2H_1 form equally), and if HH pairing is also random, then the yield of the desired bispecific hybrid antibody, $L_1H_1-H_2L_2$, is 12.5% of the total immunoglobulin. In actual fact, however, there is likely to be a homologous preference in LH pairing. If this preference were absolute, and HH pairing random, then the yield of the desired bispecific hybrid rises to 50% of the total immunoglobulin production. To maximize the chances of random HH association, we deliberately chose parental antibodies of the same subclass, IgG2a. This probably made the physical separation of the parental and recombinant antibodies more difficult, and, indeed, the two parental immunoglobulins F23.1 and 19E12 were not separable by chromatofocusing or DEAE-Sephadex chromatography. However, the two were well resolved on hydroxylapatite chromatography (Fig. 2). Since our electrophoretic analysis of the column fractions was not sufficient to estimate the degree of heterologous LH pairing, the middle peak from the chromatographic step, which contains the desired hybrid, may not be pure but may contain other undesired species such as $L_1H_1-H_2L_1$. We suspect that actual yield of the desired hybrid is somewhere between 12.5% and 50% of total immunoglobulin production.

Coating targets versus coating effectors

Liu *et al.* have reported that a chemical heteroconjugate anti-T3/anti-immunoglobulin idiotype can target the idiotype expressing human B-cell lymphoma for lysis

by a human CTL clone of unrelated specificity¹³. In this case, lysis occurred much more effectively when the target cells were precoated with the heteroconjugate than when the effector cells were precoated. We have done similar experiments *in vitro* with the purified H1.10.1.6 bispecific antibody (Table 2). In agreement with Liu *et al.*, the hybrid antibody directed lysis of SL2 (Thy 1.1⁺) target cells by OE4 effectors is more efficient when the target, rather than the effector cell, is coated with antibody. In our case, however, this difference is only roughly a factor of three. Furthermore, when both effector and target were coated, lysis occurred at the higher level (not shown in Table 2). What this implies is that the decreased level of lysis obtained by precoating the effectors is not due to a 'premature ejaculation' induced in the CTL by the anti-receptor antibody. H1.10.1.6 hybrid antibody is monovalent for anti-receptor activity and may not lead to triggering the effector nor to capping of the receptor in the absence of the targeted cell type.

Therapy *in vivo*

Hybrid antibody (anti-receptor/anti-X) targeting works extremely efficiently *in vitro*, and the hope is that it may also focus T-cell activity to the site of infections or tumors *in vivo*. In humans, an obvious choice of anti-receptor antibody is one of the many anti-T3 mAb that exist.

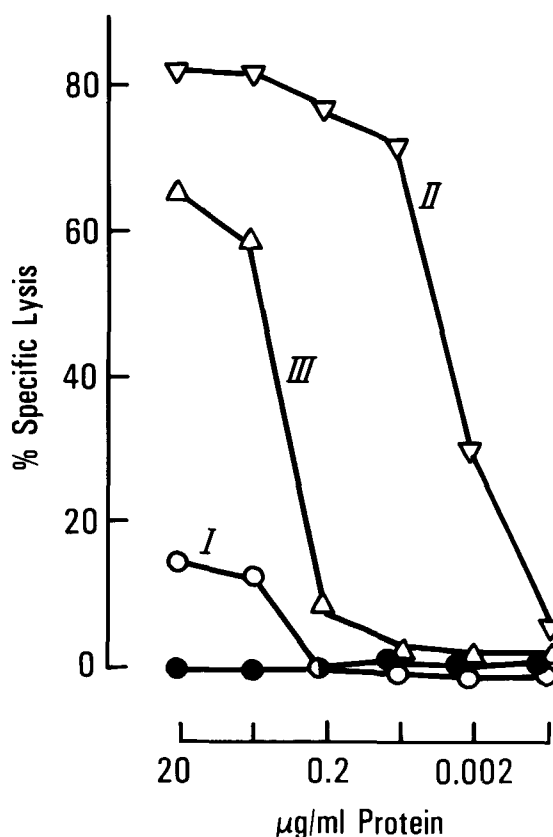


Fig. 3. Targeting activity of the HPLC peaks of H1.10.1.6 immunoglobulin. 3×10^4 OE4 cells (CTL clone H-2^b anti-H-2^d, F23.1⁺) were assayed for lysis of 1×10^4 ⁵¹Cr-labelled S.AKR cells (H-2^k, Thy 1.1) for 4 h at 37°C with no addition (●) or in the presence of decreasing amounts of HPLC purified material: Peak I (○), Peak II (▽), Peak III (△).

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Table 2. Precoating targets versus precoating effector cells with hybrid antibody

Effector CTL Clone	Effector: target ratio	Target Cells							
		EL4 (H-2 ^b , Thy 1.2)				SL2 (H-2 ^k , Thy 1.1)			
		Control	PHA	Precoat target	Precoat CTL	Control	PHA	Precoat target	Precoat CTL
OE4	5:1	0	65	1	7	0	66	67	47
OE4	1:1	0	46	1	2	-5	35	52	22
G4	5:1	0	73	1	1	-1	73	1	0
G4	1:1	0	63	1	-1	0	54	-3	-5

The HPLC purified hybrid antibody, F23.1/19E12, secreted by the H1.10.1.6 hybridoma, was used at 10 µg/ml to coat target cells or CTL clones at 37°C for 30 min. After washing, effectors and target cells were incubated together at the indicated ratios for 4 h at 37°C. Both CTL clones express Thy 1.2 and H-2^b and are specific for H-2^a antigens. OE4 expresses the β chain recognized by F23.1 mAb while G4 does not. Phytohemagglutinin (PHA) was included in the assay at 10 µg/ml.

These react with all mature T cells of helper and cytotoxic lineage. Another choice for anti-receptor antibody could be those which recognize determinants on the α,β heterodimer of a fraction of human T cells. mAb that react with 1–4% of T cells from many donors have been described^{16,17}. Since the α,β structure also seems to be shared by the two T-cell lineages, those mAb probably react with the both helpers and CTL. Since it is not clear what functional subset of T cells may cure a certain disease, it is wise to recruit both subsets. The choice of specificity for the second, targeting antibody is more difficult to make. If one intended to use this technique to focus T cells to the site of a viral infection, then it is likely that many mAbs would be available, and we should opt for ones which recognize infected cells, but not the free virus. For targeting tumors, one would hope for a pre-existing battery of tumor-specific or tumor-associated mAb.

In a murine model system to study the efficacy of hybrid antibody targeting, we have done preliminary work on controlling the growth of a transplantable AKR/J T-cell lymphoma, S.AKR (which expresses the Thy 1.1 determinant) in the closely related Thy 1.2 strain, AKR/

Cum. Our original plan was based on the assumption that S.AKR tumor cells would grow in and kill AKR/Cum mice. We planned to give a lethal dose of tumor cells to normal AKR/Cum mice and, at a later stage, to induce effector T cells in the mice with an allogeneic, H-2^b skin graft, and when the allogeneic response was high, to focus these cells to the S.AKR tumor by injecting the F23.1/19E12 hybrid antibody. However, in titrating S.AKR cells in AKR/Cum hosts, we found that normal animals could resist tumor growth. Prior irradiation of the mice allowed tumor growth leading to the death of the hosts. Thus, AKR/Cum mice, which are not truly congenic with AKR/J, can reject this tumor by a radiation-sensitive mechanism, i.e., probably a response to minor histocompatibility differences.

In the first tests of hybrid antibody targeting in this tumor/host system, we were obliged to sublethally irradiate the AKR/Cum hosts before injecting a dose of tumor cells which would lead to the death of 90% of the mice. Mice were given 400r whole body irradiation on day 0, followed by an i.v. injection of 10⁵ S.AKR cells. On day 2, the mice were split into five groups and injected i.v. with either: (1) saline; (2) F23.1; (3) 19E12; (4) a mixture of the two; or (5) the purified hybrid antibody H1.10.1.6. Mixed lymphocyte culture activated AKR/Cum cells were injected i.v. into all five groups of mice 4 h later. On day 9, all mice received an injection of allogeneic cells, and on day 12, each group received saline or antibody i.v. once more. Fig. 4 shows the survival data for the five groups of animals. If the four control groups are pooled, then 17/20 of the mice died by day 33 after tumor injection. Of the mice which received the targeting antibody, H1.10.1.6, there was only one death on day 29. Although the number of animals per group is small, this result suggests that the presence of the bispecific antibody protected the mice in a way that each parental antibody alone or as a mixture did not. Future work in this system will make use of a lymphoma line which grows progressively in unirradiated AKR/Cum mice, in which case we will have the option of recruiting effector T cells induced in the host.

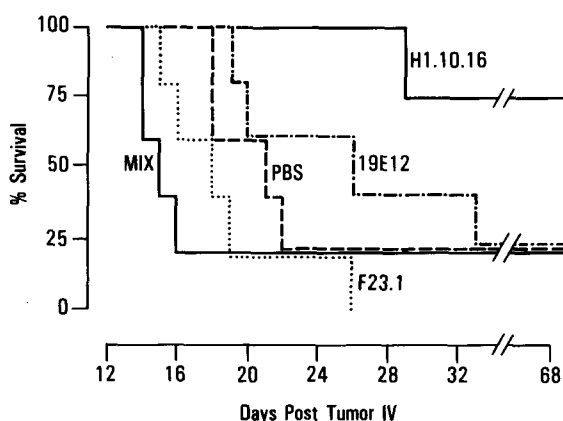


Fig. 4. Targeting a tumor in vivo. Young adult AKR/Cum female mice were irradiated on day 0 with 400r and injected i.v. with 10⁵ S.AKR (Thy 1.1) lymphoma cells 2 h later. Two days later, groups of mice were injected i.v. with saline, or 18 µg of F(ab')₂ fragments of antibodies, F23.1, 19E12, a mixture of the two, or H1.10.1.6. Later the same day, each mouse received 10⁷ AKR/Cum spleen cells i.v. which had been activated for 4 days in culture with irradiated B6 spleen cells. Irradiated B6 cells were injected i.p. on day 9 and the same antibody injections were given to the five groups on day 12.

Future work

We plan to pursue the therapeutic applications of hybrid antibodies in murine model systems. To do this, we will continue to use the anti-allotypic mAb, F23.1, which reacts with ~25% of T-cell receptors. To use anti-idiotypic mAbs for *in-vivo* work is to confine oneself

to using one particular T-cell clone and its syngeneic mouse strain. We have chemical heteroconjugates F23.1/ anti-influenza, and we are attempting to isolate a hybrid hybridoma of this specificity. Such bispecific antibodies would allow us to study the rate of clearance of virus from the lungs of mice, for example.

In murine and human systems, a great deal of preliminary studies need to be done. Amongst the questions being addressed are: What form of bispecific antibody should one use – 7S or F(ab')₂? Should one activate the effector T cells *in vitro* or *in vivo*? Will T cells home to sites of inflammation when they are coated with a bispecific antibody? The concept of using antibodies to focus T-cell function deserves to be tested thoroughly for its applications *in vivo*.

This work was performed while Uwe D. Staerz was a graduate student in the Department of Biology, University of California, San Diego, La Jolla, California 92093, and it forms a part of his Ph.D. thesis.

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The Inflammatory Reaction

by H.Z. Movat, Elsevier Biomedical Press, 1985. US\$ 96.25/Dfl 260.00 (xv + 365 pages)
ISBN 0 444 80662 8

This book attempts to analyse inflammation from a multi-disciplinary view, correlating structure and function. The emphasis is on mechanisms and on the historic development of our current view of these processes. It springs from the author's personal involvement in this field over more than three decades. The result is an interpretative overview of the inflammatory process with a strong historical perspective ranging back over the last century. This will provide a useful introductory background to young scientists or clinicians entering any field dealing with inflammation – also to the researcher who having buried himself in one small corner of the field now wants to set that in a wider perspective. This is definitely a view of the forest but is sufficiently well referenced for those who need to find where to get a closer look at the individual trees.

The book starts with an historical review which is a brief dedication to the giants of the past, indicating the solid basis which they gave to the subject and on which modern tools such as electron microscopy or en-

zyme analysis have built. This perspective is continued in each chapter, while the experimental method is stressed throughout, so that not only the historical data but the excitement and the rigours of the early experiments in inflammation are conveyed to the reader. The basic physiology of the microcirculation, the lymphatics and the inflammatory cells are dealt with rather than just a description of the changes in inflammation. This allows greater understanding of mechanisms already discovered as well as providing an essential broad background for looking at future developments in this field.

The book may appear old-fashioned at first although the extensive references go up to 1985. The use of the historical background to each of the modern concepts in my view is successful and allows a more integrated view of inflammation. For example the author reinterprets the older models of chemical and thermal inflammation in the light of modern ideas of what happens in 'natural' inflammation induced by Gram negative bacteria or immune complexes, which may have more biological relevance. Again the historical observations of polymorph adhesiveness to microvascular endothelium only at the site of injury is

used to lead up to the new data on the role of local IL-1 production by endothelial cells as the initiating factor in cell sticking.

The book does not just look back but points to unresolved issues needing examination. Thus the idea is mooted that differences between endothelial cells at different sites may well have important clues for the site of specific inflammatory diseases in a way in which blanket ideas of inflammation as a unitary process cannot provide. The overall result is that of a classical text which refuses to get involved in the latest twist of arachadonate or oxidative metabolism but sets them firmly in a wider context. Its broad biological rather than detailed biochemical bias appealed to me though this may disappoint the reader looking for a 'state of the art' review of the latest data. Perhaps this organic view is essential to assess the real relevance of all such observations, even if the current excitements in research seem to be at a subcellular or molecular level. The discussion of chronic inflammation as both a clinical concept (failure to heal) and as a broader pathophysiological concept involving specific cell types, is timely.

In a volume this size dealing with such a multi-faceted subject, it is easy to find omission of points of