Name:	**C3d**
Catalog Number:	**A117**
Sizes Available:	100 µg/vial
Concentration:	1.0 mg/ml (see Certificate of Analysis for exact conc.)
Form:	Frozen liquid
Purity:	>95% by SDS-PAGE
Buffer:	10 mM Sodium phosphate, 145 mM NaCl, pH 7.3
Extinction Coeff.	$A_{280\ nm}$ = 1.10 at 1.0 mg/ml
Molecular Weight	33,800 Da (single chain)
Presevarive:	None, 0.22 µm filtered
Storage:	-70^{o}C or below. Avoid freeze/thaw.
Source:	Normal human serum (shown by certified tests to be negative for HBsAg and for antibodies to HCV, HIV-1 and HIV-II).
Precautions:	Use normal precautions for handling human blood products.
Origin:	Manufactured in the USA.

General Description

C3d is derived from human C3 after a series of proteolytic cleavages. Trypsin-like enzymes (such as plasmin, thrombin, elastase, etc.) cleave iC3b separating C3c from C3dg (38,900 Da) and C3dg is further digested releasing C3g (5,100 Da) to leave C3d. iC3b (inactivated C3b) is derived from C3b. C3b itself is produced by all three pathways of complement (Law, S.K.A. and Reid, K.B.M. (1995)) when native C3 is cleaved releasing C3a.

C3d is prepared at CompTech from purified human C3. The C3 is converted to C3b by treatment with the natural human C3 convertase and this C3b is converted to iC3b by treatment with complement factors H and I. Finally, this iC3b is cleaved with trypsin to yield C3d. The C3d made by CompTech is made from soluble C3b. If the C3b precursor was attached to a surface, such as occurs during complement activation, the iC3b, C3dg and C3d fragments would remain covalently attached to that surface. C3c is released into the fluid phase. The C3d sold by CompTech is not capable of attaching to a surface. This can only occur during complement activation on a surface. Surface-bound C3d is linked to the target through an ester or an amide covalent bond. Ester bonds are unstable resulting in the gradual release of C3d from the particle.

Physical Characteristics & Structure

C3d molecular weight: 33,800 daltons. C3d is a single chain protein. Human C3d is not glycosylated. The 3-D structure of C3d has been reported (Nagar, B. et al., (1998); Gros P. et al., (2008)). The C-terminal arginine of C3d may or may not be removed by serum carboxypeptidases leading to heterogeneity.

Function

Surface-bound C3d and its precursors C3dg and iC3b are recognized by complement receptor 2 (CR2, CD21) present on mature B cells and follicular dendritic cells which use these ligands to stimulate phagocytosis and antigen presentation to cells of the adaptive immune system (Dodds, A.W. and Sim, R.B. editors (1997); Morley, B.J. and Walport, M.J. (2000)). The interaction between C3d/C3dg/iC3b and CR2 is essential

for a normal antibody response to antigens to which C3d/C3dg/iC3b are attached. This forms an important link between the innate immune response of complement and adaptive immune response to infectious organisms. Tagging pathogens or antigens with the ligands C3d, C3dg or iC3b as the result of complement activation can dramatically stimulate the immune response to those antigens (Dempsey PW, et al. (1996))

Assays

There are no functional assays for C3d. SDS gels and Western blots can be used to determine the chain structure of the protein. C3d does bind to the B-cell receptor CR2, but the affinity between a single C3d and CR2 is weak. This interaction is best observed with particles coated with many C3d molecules or with cross-linked C3d multimers (Carter, R.H. and Fearon, D.T. (1989)).

In vivo

The primary method of formation of C3d/C3dg in blood is the cleavage of iC3b by factor I induced by the interaction with CR1 (a membrane-bound receptor on human erythrocytes). This cleavage is not induced by factor H, only by CR1. This second cleavage by factor I results in the separation of C3dg from C3c (Dodds, A.W. and Sim, R.B. editors (1997). In the absence of CR1 conversion of iC3b to C3dg is a very slow process (6 days at 37^{o}C (Vik, D.P. and Fearon, D.T., (1985))). Formation of C3d from C3dg in blood is also a slow process. Because of the excess of protease inhibitors in plasma there is very little free thrombin, plasmin, etc. existing as active proteases so most C3dg remains as C3dg. Only at sites of inflammation or with accompanying blood clotting will complement activation yield large amounts of C3d.

Regulation

Most C3dg/C3d and the particles to which they are attached are cleared by interaction with receptors and thereby removed from tissues or from circulation.

Genetics

Human chromosome location of the C3 gene is 19p13.3. The mouse chromosome location is chromosome 17 and the rat chromosome 9. Accession numbers K02765 (human) and K02782 (mouse). Human C3 genomic structure: the gene spans 41 kb with 41 exons

Precautions/Toxicity/Hazards

The source of this protein is human serum, therefore precautions appropriate for handling any blood-derived product must be used even though the source was shown by certified tests to be negative for HBsAg, HTLV-I/II, STS, and for antibodies to HCV, HIV-1 and HIV-II. MSDS sheet is available upon request.

References

Carter, R.H. and Fearon, D.T. (1989) Polymeric C3dg primes human B lymphocytes for proliferation induced by anti-IgM. J. Immunol. 143:1755-1760.

Dempsey, P.W., Allison, M.E., Akkaraju, S., Goodnow, C.C. and Fearon, D.T. (1996). C3d of complement as a molecular adjuvant: bridging innate and acquired immunity. Science 271:348-350.

Dodds, A.W. and Sim, R.B. editors (1997) Complement. A Practical Approach (ISBN 019963539) Oxford University Press, Oxford.

Gros, P., Milder, F.J., and Janssen, B.J. (2008) Complement driven by conformational changes. Nat Rev Immunol. 8:48-58.

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Vik, D.P. and Fearon, D.T., (1985) Neutrophils express a receptor for iC3b, C3dg, and C3d that is distinct from CR1, CR2, and CR3. J Immunol 134:2571-2579.

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