



# Measles Virus is Associated with Hodgkin Lymphoma and Additional Tumors-A Never Ending Story

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## ARTICLE HISTORY

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### “Ancient History” Microbes and Cancer

- About 15% of human cancers-through to arise by mechanisms that involve **Viruses**, bacteria or parasites.
- Evidence for their involvement partly from the detection of the agents in biopsies....
- ...and partly from animal and epidemiological studies.

### The Biologic Agent (Virus) is Usually Responsible for only a Limited Number of Steps in the Initiation/Progression of Cancer

- In many cases the precise role of cancer-associated virus is hard to decipher due to the long delay from the initial viral infection!
- The number of individuals infected by the virus is much larger than that with virus-associated cancers
- Therefore, viruses must act in conjunction with other factors.

### Chronic Inflammation and Cancer

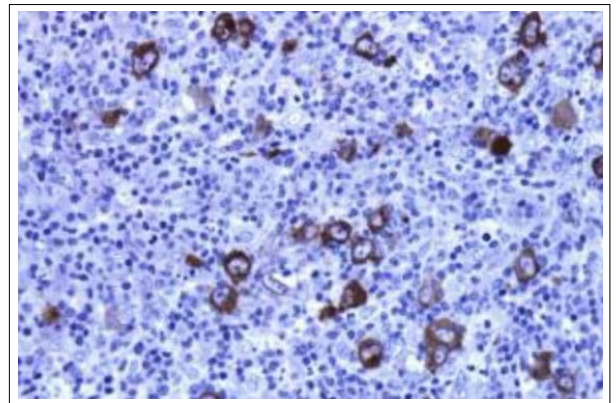
- In some cases of unresolved chronic inflammation, the immune response becomes maladaptive, promoting tumorigenesis.
- A regenerative process supported by numerous bioactive mediators, promote cell survival, tissue remodeling and angiogenesis
- The mediators also cause genomic stress and mutations

### Classic Hodgkin Lymphoma

- Classic Hodgkin lymphoma is a cancer of lymph nodes and of the immune system
- Infrequent, but one of the most frequent cancers of young adults (15-34)
- As a very few cancer cells in tumor mass

- Very good response to treatment (80% and more are cured)

### EBV/Latent Membrane Protein-1 Expression in Classic Hodgkin Lymphoma



### Epidemiologic Correlation of EBV in Classic Hodgkin Lymphoma

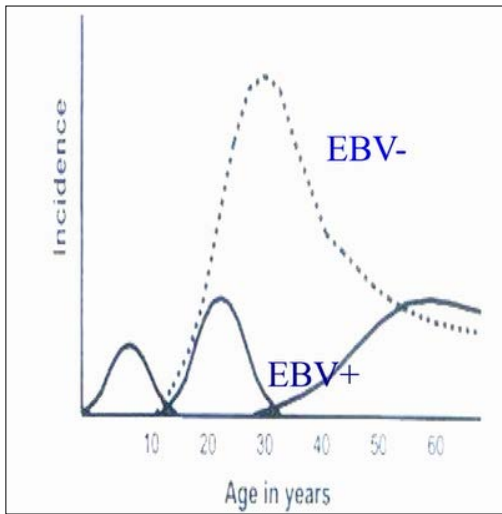
- The association of EBV in cHL varies from 17% in some industrialized countries to 100% in a few developing countries
- EBV expression varies with gender:
  - Male patients are more likely to be EBV positive than are female patients
- EBV expression varies with histologic subtype:
  - Mixed cellularity (32-96%)
  - Nodular sclerosis (10-50%)

### Pathogenesis of EBV-Related cHL

- EBV-associated NF- $\kappa$ B activation may lead to cell proliferation, inflammation and apoptosis arrest
- EBV/LMP1 has a Bcl-2-like effect and causes apoptosis resistance

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- Children mostly EBV-positive males with MCHL
- Young adults (both sexes) mostly EBV-negative NSHL
- Young adults with previous mononucleosis, EBV positive
- Older adults mainly EBV positive males with MCHL



Thus, EBV may account for only part of cHL cases

**Young-adult cHL patients, mainly women, who are those most expected to be related with late exposure to a common infection (according to hallmark epidemiologic studies) have the lowest link to EBV.**

None of the additional viruses, considered so far as possible candidates, have been found in cHL tissues

**In Favor of a Measles Virus-Associated with Classic Hodgkin Lymphoma**

- Measles virus is lymphotropic
- It also shows tropism for the oropharyngeal epithelium epithelium
- Measles infection is regularly followed by transient immune deficiency
- Multinucleated giant cells are seen in measles and in cHL
- Since 1963, following anti-MV vaccination, measles infection occurs more often after age 5

**We have proposed the measles virus as a possible biologic agent related to classic hodgkin lymphoma at least in young adult patients.**

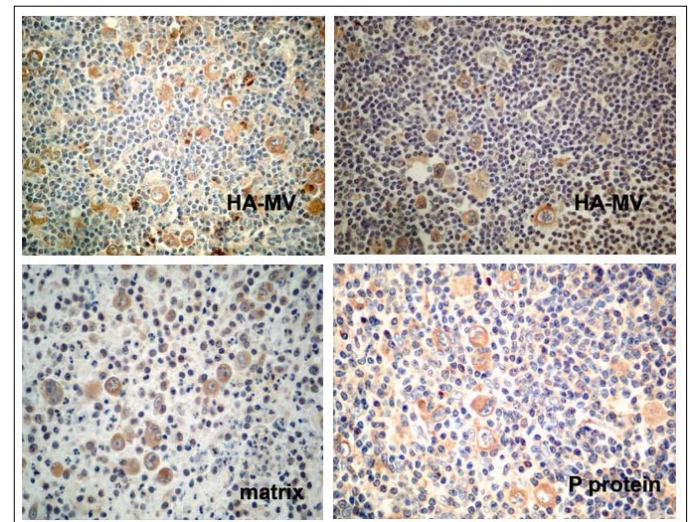
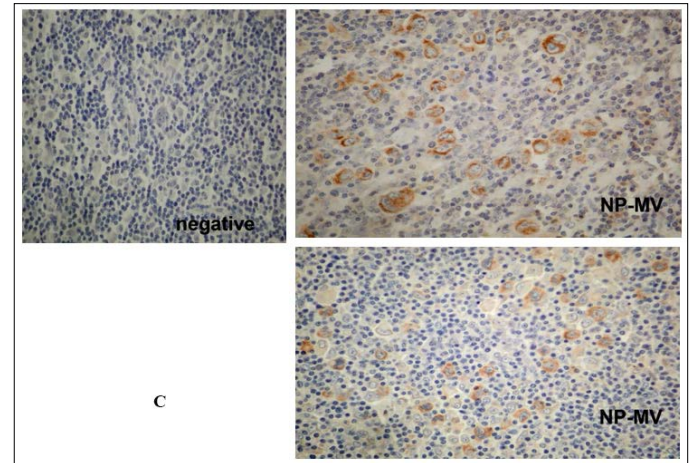
**Patients and Methods**

- 154 biopsies from untreated cHL patients
- IHC study in paraffin sections-avidin-biotin peroxidase complex method
- Reverse transcriptase-polymerase chain reaction on total

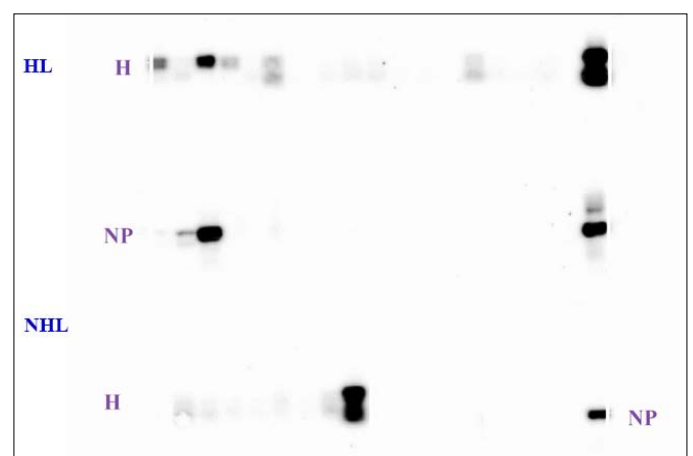
RNA from frozen & paraffinembedded tissues

- Products of nested PCR were blotted
- In situ hybridization according to ogata et al

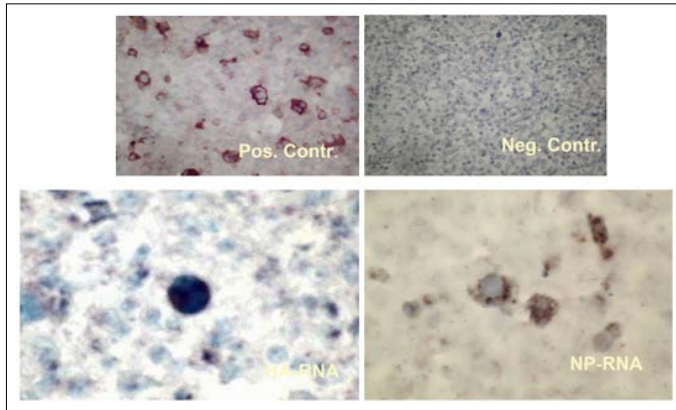
**Immunohistochemistry**



**Southern Blot**



### In Situ Hybridization



#### Results

- 82(54%) were positive for two MV antigens or more
- HA-MV RNA+(4/15); NP-MVR NA+ (2/16)-by southern blot
- MA-MV RNA+(2/7); NP-MV RNA+ (8/21) by in situ hybridization

#### Hodgkin Lymphoma-Association with MV

		MV+	MV-	P value
		N (%)	N (%)	
Total		82(54.3)	69(45.7)	
Gender	F	40(64.5)	28(35.5)	.036
	M	42(47.2)	47(52.8)	
Type	NS	58(68.2)	27(31.8)	.0013
	MC	21(40.4)	31(59.6)	
CD15	pos	77(59.7)	52(40.3)	.015
	neg	4(26.7)	11(73.3)	
P53	pos	72(61.5)	45(38.5)	.017
	neg	5(31.3)	11(68.8)	

#### Hodgkin Lymphoma-Association with Measles and Epstein-Barr Viruses

		MV+; LMP+	MV-; LMP+	P value
Type	NS	45(86.5)	7(13.5)	.036
	MC	11(44)	14(56)	
GStage	I-IIA	22(84.6)	4(15.4)	.036
	IIB-IVB	22(57.9)	16(42.1)	
BCL-2	pos	31(93.9)	2(6.1)	.0013
	neg	27(54)	23(46)	
MDM-2	pos	36(87.8)	5(12.2)	.015
	neg	18(54.5)	15(45.5)	
Apop Index	<median	31(81.6)	7(18.4)	.017
	>median	22(42.1)	16(57.9)	

### Multivariable Logistic Regression Model of Association of Prognostic Factors with Dying of HL in 89 Patients

Factor	Odds Ratio	95% CI	P Value
Gender	1.32	.34-5.18	.69
Stage	13.67	2.3-80.5	.004
Bcl-2	.34	.08-1.44	.34
MV+; EBV-	10.05	.98-103.47	.05
MV-; EBV+	1.53	.07.35.44	.79
MV-; EBV-	15.2	1.05-220.3	.04

#### Comments

- We have shown an association between MV and HL (though causality was not proven!) ...
- ... supported by epidemiologic data on brain and spinal cord tumors, as well as cHL, following exposure to MV around the time of birth
- Sporadic cHL regression is reported after measles or MV vaccination
- Childhood infections (including measles!) protect from cHL
- Preliminary clinical correlations show more patients with early stages among MV positive expression in cHL patients
- **By contrast, positive MV- expression in cHL seem to be associated with a poor prognosis. It is not excluded that the advantage conferred to the above-mentioned parameters is nullified by the expression of MV**
- Isolated MV expression and absence of both EBV and MV expression are independent poor prognostic factors
- Positive EBV expression is related with a longer survival
- **The longest survival is related with the positive expression of both viruses**

#### "OLD HISTORY"

The German Connection-E. Maggio, R. Küppers

- In order to evaluate our results, Maggio et al selected 18/44 of their own cHL fresh frozen tissues for their high-quality RNA and good HRS cell morphology. They used UV-laser beam single cell micro dissection
- About 100 cells were pooled for each experiment, RNA was extracted and RT-PCR performed with primers from three MV genes

#### Results

- The CHL-derived cell lines - L428, L1236, KMH2, HDLM2 were negative for the 3 MV transcripts
- The 18 German cases did not show evidence of any of the three viral transcripts. GAPDH housekeeper control!) Gene showed strong evidence of RNA

#### The Israeli Connection

- We sent 22 of our fresh-frozen CHL tissues to Germany, chosen for **their positive IHC staining with MV antigens**
- But only 7 of these were selected for high quality RNA and classic HRS cell morphology in Essen

- These were all found by Maggio et al, to be negative for the various MV transcripts studied
- Five of twelve German cases were found by us to show MV antigens expression in HRS cells
- A similar discrepancy in favor of IHC obtained also in our patients

**Comments on the Debate**

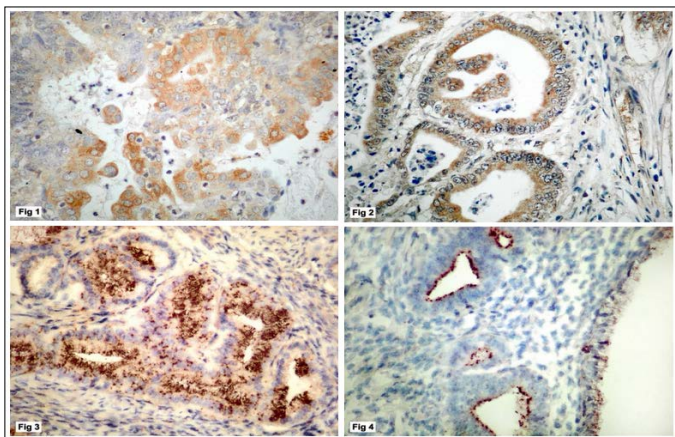
- In the German study few but highly selective cases were studied using different, more sophisticated, methods than ours
- Classic HL tissues are rich in ribonucleases (in eosinophils!). MV-RNAs, as expected, were of low abundance
- GAPDH with its abundant RNA may not be an adequate choice for a housekeeping gene in this experiment
- Our 7 cases studied in Germany were all MC-HL, most were EBV positive. We had studied 5 for MV-RNA. They were faint (2) or negative (3)
- A possible additional evidence of a role for MV in carcinogenesis is that MV proteins are capable of interacting and stabilizing the Pirh2 protein, an ubiquitin E3 ligase by preventing its ubiquitination
- Pirh2 negatively regulates p53 by targeting it for degradation in the proteasome

**"Recent History": MV Association with Other Cancers:**

**Endometrial Carcinoma and Measles Virus**

- We studied 49 cases of EC, 36 with known follow-up
- 26/36 (72%) expressed MV antigens in tumor cells, mainly in type I EC
- 16/21 were positive for MV-RNA by ISH
- When type II EC was allied with MV- deeper myometrial invasion and a higher mortality were evident
- Benharroch et al. Eur J Obstet Gynecol Reproductive Biol 2009;147:206-209

**Endometrial Carcinoma and Measles Virus**



**Lung Cancer and Measles Virus Antigens Expression**

- Pirh2, the E3 ubiquitin ligase is expressed in lung cancer and inhibits p53. J Natl Cancer Inst 2004; 96:1718-21
- MV-P protein inhibits the function of Pirh2
- 65 NSCLC diagnosed during 10 years
- 54/65 were positive for MV antigens associated with older patient age and positive Pirh2 expression as well as with improved survival
- Sion-Vardy et al. Exp Lung Res 2009; 35:701-12

**Breast Cancer and the Measles Virus**

- 64% of 131 cases were positive for MV antigens by IHC
- MV antigens expression was associated with ER (p=.018); with Ki-67<40% (p=.029); with low or intermediate grade (p=.037); with age<50 (p-.039); with p53 over-expression (p=.049)

**Breast Cancer, Conclusions**

- Evidence of MV antigens expression in a high proportion of breast cancers
- MV association with younger age, lower histologic grade and p53 overexpression suggests it may play a role in the development of breast cancer
- Ariad et al. Anticancer Res 2011;31: 913-920

**"NEW FINDINGS": Melanoma and Measles Virus**

Antigen	Level	Melanom a	Benignnevi	P Value
NP-Mv	pos	46	23	
	neg	10	1	0.16
P-protein	pos	19	10	
	neg	36	12	0.37
HA-MV	pos	19	7	
	neg	3	1	1.00
PirH-2	pos	44	19	
	neg	12	6	0.79

**Are there MV-Negative Cancers?**

- The non-Hodgkin lymphomas tested with cHL (25) were negative for MV antigens. But ALKI-positive anaplastic large cell lymphomas were positive.
- Seminomas showed in a few cases background staining for MV antigens, but they were negative.

**Additional Cancers Negative for Measles Virus**

Prof. Samuel Ariad - communication:

- Glioblastomas
- Mesotheliomas
- Additional study in isolated tumors:
- Merkel cell tumors
- Hepatocellular carcinoma
- MMTT
- Desmoid tumor

**A Breakthrough???**

Apoptosis of HRS cells in cHL revisited. Benharroch et al. APMIS 2010; 118:339-345

- We reviewed the role of apoptosis in HRS cells of classic HL, **in the light of conflicting evidence**. We found that HRS cells showed inhibition of apoptosis in 55% of the 217 cases only.
- It is also suggested that NF-κB (p65) and LMP1/EBV do not correlate with apoptosis inhibition, in contrast with the consensus view

**Apoptosis of HRS Cells (2)**

- The most significant association of HRS cell apoptosis was with p53, the negative expression of which was related with a high apoptotic index (p=.001)
- We analyzed the relationship between positive MV expression and factors related with apoptosis and found associations with apoptotic index less than median (p=.005); with Mdm2+ (p=.028): with IxB+ (p.0001).

**Apoptosis of HRS Cells-Conclusions**

- Apoptotic index lower than median was related with positive MV expression, as was a positive MDM2 expression
- Viruses have a propensity to modulate apoptotic mechanisms in the infected cell
- We believe that our demonstration of a pathogenic mechanism involving MV in CHL may favor a causal role for MV, at least in cHL

**Provisional Overall Conclusions**

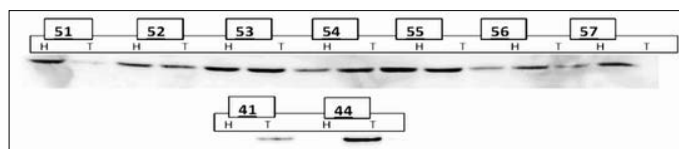
- We found a relation between MV expression and several malignancies
- In CHL this might even be causally related with apoptosis modulation as the supportive mechanism
- Findings are probably sustained by an increased CHL morbidity related with a raised measles occurrence in young adults, in Israel (Ariad et al, IMAJ 2010), in Quebec and in Connecticut. This may be due to frequent outbursts of measles

**Recent Confirmation of MV Expression in Cancer**

- A Western blot was carried out of 18 non-small cell lung cancer frozen tissues, as well as of non-malignant lung tissues thereof.
- We used an anti-P-MV antibody, 9H4 from Santa Cruz Biotechnology.

Most cases were Adenocarcinomas; 84.69% were positive for MV, with remote 83.3% positive normal lung tissue

**Western Blot with Non-Small Cell Lung Tumors (T-Tumor; H-Normal Lung)**



**Table: Tissue Micro-Array using a Phosphoprotein-MV Antibody**

Organ	Positive Staining %			Negative Staining %		
	Nucleus	Cytoplasm	Stroma	Nucleus	Cytoplasm	Stroma
Lung	10 (52.6%)	2 (11.1%)	0	9 (47.4%)	16 (88.9%)	18 (100%)
Colon	13 (68.4%)	7 (36.8%)	4 (23.5%)	6 (31.6%)	12 (63.2%)	13 (76.5%)
Stomach	14 (73.7%)	4 (21%)	0	5 (26.3%)	15 (79%)	19 (100%)
Breast	15 (75%)	10 (50%)	2 (10%)	5 (25%)	10 (50%)	18 (90%)

**Atypical Measles Syndrome**

Atypical Measles Syndrome (AMS) differs from classic measles by almost each and every feature.

AMS probably involves immune complexes.

AMS is rare, m/p due to under diagnosis.

AMS is related with immune waning, and exposure to a wild type MV infection. Thus it may be linked with a persistent MV infection. Hence, an association with carcinogenesis should be researched.